

Annual Report to Congress – 1998 Appendices

Appendix A: Federally Funded Research Projects

Appendix A: project index

Study #	Study Title
<u>Department of Defense</u>	
DoD-1	Naval Health Study Program
DoD-1A	Epidemiologic Studies of Morbidity Among Gulf War Veterans: A Search for Etiologic Agents and Risk Factors; Study 1: A Study of Symptoms Among 1500 Seabees
DoD-1B	Epidemiologic Studies of Morbidity Among Gulf War Veterans: A Search for Etiologic Agents and Risk Factors; Study 2: A comparative study of hospitalizations among Active-duty personnel who participated in the Gulf War and similar personnel who did not.
DoD-1C	Epidemiologic Studies of Morbidity Among Gulf War Veterans: A Search for Etiologic Agents and Risk Factors; Study 3: A comparative study of pregnancy outcomes among Gulf war veterans and other active-duty personnel.
DoD-1D	Epidemiologic Studies of Morbidity Among Gulf War Veterans: A Search for Etiologic Agents and Risk Factors; Study 4: Adverse Reproductive Outcomes in Gulf War Veterans
DoD-1E	Epidemiologic Studies of Morbidity Among Gulf War Veterans: A Search for Etiologic Agents and Risk Factors; Study 5: Seabee Health Study.
DoD-1F	Epidemiologic Studies of Morbidity Among Gulf War Veterans: A Search for Etiologic Agents and Risk Factors; Study 6: A Comparison of Nonfederal Hospitalization Experience Among Veterans in California who have separated from active service.

	PGW vs. EV.
DoD-1G	Epidemiologic Studies of Morbidity Among Gulf War Veterans: A Search for Etiologic Agents and Risk Factors; Study 7: Prevalence of Congenital Anomalies Among Children of Persian Gulf War Veterans.
DoD-2	Physiological and Neurobehavioral Effects in Rodents from Exposure to Pyridostigmine, Fuels, and DEET
DoD-4	The General Well-Being of Gulf War Era Service Personnel from the States of Pennsylvania and Hawaii: A Survey
DoD-6	Program DoD-6
DoD-6A	Combat Stress Pharmacotherapy
DoD-6B	Combat Stress Diagnosis, PTSD Prevention
DoD-7A	Health Risk Assessment of Embedded Depleted Uranium: Behavior, Physiology, Histology, and Biokinetic Modeling
DoD-7B	Carcinogenicity of Depleted Uranium Fragments
DoD-8	Program DoD-8
DoD-8A	Serologic Diagnosis of Viscerotropic Leishmaniasis (VTL)
DoD-8B	Development of a Leishmania Skin Test Antigen (LSTA)
DoD-9	Identification of the Genetic Factors Which Control Tropism in Leishmania
DoD-10	Pyridostigmine Synergistic Toxicity Study
DoD-11	Male/Female Differential Tolerances to Pyridostigmine Bromide
DoD-12	Forward Deployable Diagnostics for Infectious Diseases
DoD-13	Effects of Persian Gulf War Service on Military Working Dogs

DoD-14	Risk Factors Among US Army Soldiers for Enrolling on the Department of Veterans Affairs Gulf War Registry
DoD-15	Comparative Mortality Among US Military Personnel Worldwide During Operations Desert Shield and Desert Storm
DoD-16	Kuwait Oil Fire Health Risk Assessment
DoD-17	Retrospective Studies Involving Military Use of Pyridostigmine as a Pretreatment for Nerve Agent Poisoning
DoD-18	Kuwait Oil Fires Troop Exposure Assessment Model (TEAM)
DoD-19	Persian Gulf Veterans Health Tracking System
DoD-20	A Statistical Study Correlating the Reported Cases of Gulf War Syndrome to Battlefield Locations of Afflicted US Army Personnel During the Iraq-Kuwait War; Part 1: Method to Related Troop Deployment and the Reported Cases of Gulf War Syndrome and Probable Incidence of Maladies Defined by ICD-9-CM
DoD-21	Study of Variability In Pyridostigmine Inhibition of Blood Cholinesterases in Healthy Adults and Individuals With Symptoms Following Participation in Operation Desert Storm
DoD-22	Chronic Organophosphorous Exposure and Cognition
DoD-23	Acute and Long-Term Impact of Deployment to Southwest Asia on the Physical and Mental Health of Soldiers and their Families
DoD-30	Epidemiological Studies Persian Gulf War Illnesses, PG Women's Health Linkage Study
DoD-31	Dysregulation of the Stress Response in the Persian Gulf Syndrome
DoD-32	Neuropsychological Functioning in Persian Gulf Era Veterans
DoD-33	Effects of Pyridostigmine in Flinders Line Rats Differing in

	Cholinergic Sensitivity
DoD-34	Characterization of Emissions from Heaters Burning Leaded Diesel Fuel in Unvented Tents
DoD-35	Feasibility of Investigating Whether There is a Relationship Between Birth Defects and Service in the Gulf War.
DoD-36	Fatigue in Persian Gulf Syndrome-Physiologic Mechanisms
DoD-37	Neurobehavioral and Immunological Toxicity of Pyridostigmine, Permethrin, and DEET in Male and Female Rats
DoD-38	Diagnostic Antigens of <i>Leishmania tropica</i>
DoD-39	A Controlled Epidemiological and Clinical Study into the Effect of Gulf War Service on Servicemen and Women of the United Kingdom Armed Forces
DoD-40	Psychological and Neurobiological Consequences of the Gulf War Experience
DoD-41	Evaluation of Muscle Function in Persian Gulf Veterans
DoD-42	The Symptomatic Persian Gulf Veterans Protocol: An Analysis of Risk Factors with an Immunologic and Neuropsychiatric Assessment
DoD-44	Investigation of Seminal Plasma Hypersensitivity Reactions
DoD-45	Physical and Emotional Health of Gulf War Veterans Women
DoD-46	Exploratory Data Analysis with the CCEP Database
DoD-47	Study of Mycoplasmal Infections in Gulf War Veterans
DoD-48	Assessment of Genomic Instability via Chromosome 7 Inversion Frequency in a Gulf-War Syndrome Cohort vs Selected Control Groups

DoD-49	Diagnosis and Dosimetry of Exposure to Sulfur Mustard: Development of Standard Operating Procedures and Exploratory Research on Protein Adducts
DoD-50	Toxicokinetics of O-Ethyl S-(2-Diisopropylaminoethyl) Methylphosphonothioate [(+)-VX] in Rats, Hairless Guinea Pigs and Marmosets - Identification of Metabolic Pathways
DoD-51	Transgenic Engineering of Cholinesterases: Tools for Exploring Cholinergic Responses
DoD-52	Female Gender and Other Potential Predictors of Functional Health Status Among Persian Gulf War Veterans
DoD-53	Long-Term Effects of Subclinical Exposures to Sarin
DoD-54	Assessment of Subchronic Neurobehavioral and Neuropathologic Effects in Rats
DoD-55	Low-Level Exposure to GB Vapor in Air: Diagnosis/Dosimetry, Lowest Observable Effect Levels, Performance-Incapacitation, and Possible Delayed Effects
DoD-56	Low-Level Sarin Neurotoxicity and Its Modulation by Pyridostigmine
DoD-57	Physiologic Effects of Stress in Gulf War Veterans
DoD-58	Illness Among Persian Gulf War Veterans: Case Validation Studies
DoD-59	Pyridostigmine-induced Neurodegeneration: Role of neuronal Apoptosis
DoD-60	Butyrylcholinesterase Genetic Variants in Persons with Gulf War Illness
DoD-61	Neurophysiologic and Neuropathologic Effects in Monkeys of Low Level Exposures to Sarin, Pyridostigmine, Pesticides, and

	Botulinum Toxoid
DoD-62	arin and Pyridostigmine Interaction under Physical Stress: Neurotoxic Effects in Mice
DoD-63	PGW Veterans: Epidemiological and Clinical Evidence for Residual Organophosphate Neurotoxicity
DoD-64	Individual Differences in Neurobehavioral Effects of Pyridostigmine
DoD-65	Multi-disciplinary Pathophysiologic Studies of Neurotoxic Gulf War Related Syndromes Leading to Diagnosis and Treatment
DoD-66	Testing for Mycoplasmal Infection Replicability of Nucleoprotein Gene Tracking and Forensic Polymerase Chain Reaction
DoD-67	Antibacterial Treatment Method Based Upon the Excretion of Dead and Decaying Spherical Bacteria
DoD-69	Five Year Follow-Up of Arm Personnel Exposed to Chemical Warfare Agents
DoD-70	War Syndromes from 1900 to the Present: Symptom Patterns and Long-term Health Outcomes
DoD-71	A Comparison of Post Deployment Hospitalization Between Vietnam and Gulf War Veterans
DoD-72	Long-term Effects of Subchronic Exposure to Sarin, Alone and with Stress or Other Chemicals
DoD-73	Post-deployment Morbid Stress, Behavior and Health: Developing a Model for Predicting Morbidity, Mortality, and other Adverse Outcomes
DoD-74	Relationship of Stress Exposures to Health in Gulf War Veterans
DoD-75	Toxic Interactions of Prophylactic Drugs and Pesticides

DoD-76	Evaluations of Immunotoxicity due to Concurrent Exposure to DEET, Pyridostigmine, and JP-8 Jet Fuel
DoD-77	Percutaneous Absorption of Chemical Mixtures Relevant to the Gulf War
DoD-78	Experimental Models of Gulf War Syndrome
DoD-79	Time Course of Stress-induced Impairment of Blood Brain Barrier
DoD-80	Molecular Regulation of Corticosteroid Receptor Expression in Stress-Responsive Cells
DoD-81	Immunotoxicity due to Coexposure to DEET, Pyridostigmine, and Stress
DoD-82	Feasibility of Developing a Registry of PTSD Affected Veteran Sib Pairs
DoD-83	Risk for Stress-related Substance Abuse: the Effects of Family History of Alcoholism
DoD-84	Psychobiologic Alterations in Persian Gulf War Veterans with and without PTSD
DoD-85	CNS Cytokines and CRH in Gulf War Veterans with Multiple Unexplained Symptoms
DoD-86	Effects of Combat Stress on Structure and Function of the Hippocampus
DoD-87	Measurement and Validation of Psychosocial Risk and Resilience Factors Accounting for Physical and Mental Health and Health-Related Quality of Life among PGWVs
DoD-88	Clinical Relevance of novel Immunological Markers in PTSD
DoD-89	Limbic Blood Flow and Opiate Receptor PET in Posttraumatic Stress Disorder

DoD-90	SPECT Benzodiazepine Receptor and MR Imaging in PTSD
DoD-91	Neurological and Circadian Substrates of PTSD-like Behaviors
DoD-92	Traumatic Experiences Persistently Enhance Cue-dependent Learning: Toward an Animal Model of Chronic Stress and Posttraumatic Stress Disorder
DoD-93	Troops Exposed to New Agents at Aberdeen Proving Ground - Follow Up Investigation
<u>Department of Health and Human Services</u>	
HHS-1	Health Assessment of Persian Gulf War Veterans from Iowa
HHS-2	Disease Cluster in a Pennsylvania Air National Guard Unit, EPI-AID 95-18
HHS-3	Biomarkers of Susceptibility and Polycyclic Aromatic Hydrocarbon (PAH) Exposure in Urine and blood Cell DNA from U.S. Army Soldiers Exposed to Kuwaiti Oil Well Fires
HHS-4	Suspected Increase of Birth Defects and health Problems Among Children Born to Persian Gulf War Veterans In Mississippi
HHS-5	Cognitive Function and Symptom Patterns in Persian Gulf Veterans
HHS-6	Defining Gulf War Illness
HHS-7	Immunotoxicity of Dermal Permethrin and Cis-Urocanic Acid
HHS-8	Strategy to Identify Non-Additive Response to Chemical Mixtures
<u>Department of Veterans Affairs</u>	
VA-1	Mortality Follow-up Study of Persian Gulf Veterans, First Update
VA-2	National Health Survey of Persian Gulf Veterans

VA-2A	VA National Survey of Persian Gulf Veterans - Phase I
VA-2B	VA National Survey of Persian Gulf Veterans - Phase II
VA-2C	VA National Survey of Persian Gulf Veterans - Phase III
VA-3	Use of Roster of Veterans Who Served in Persian Gulf Area
VA-4	Boston Environmental Hazards Research Center Program
VA-4Core	Boston Environmental Hazards Research Center Program
VA-4A	Evaluation of Cognitive Functioning of Persian Gulf Veterans
VA-4B	Evaluation of Neurological Functioning in Persian Gulf Veterans
VA-4C	Gulf War And Vietnam Veterans Cancer Incidence Surveillance
VA-4D	Evaluation of Respiratory Dysfunction Among Gulf War Veterans
VA-4E	The Aromatic Hydrocarbon Receptor (AhR) as a Biomarker of Susceptibility
VA-4F	Validity of Computerized Tests
VA-5	East Orange Environmental Hazards Research Center Program
VA-5Core	East Orange Environmental Hazards Research Center Program
VA-5A	Health and Exposure Survey of Persian Gulf Veterans
VA-5B	Physiological and Psychological Assessments of Persian Gulf Veterans
VA-5C	Effects of Exertion and Chemical Stress on Persian Gulf Veterans
VA-5D	Effects of Genetics and Stress on Responses to Environmental Toxins
VA-6	Portland Environmental Hazards Research Center Program

VA-6Core	Core Program: Portland Environmental Hazards Research Center: Environment, Veterans Health and the Gulf War Syndrome. Core Project for Clinical and Epidemiology Research
VA-6A	Psychosocial, Neuropsychological and Neurobehavioral Assessment (Project I)
VA-6B	Clinical and Neuroendocrine Aspects of Fibromyalgia (Project II)
VA-6C	Neurotoxicity of Environmental Pollutants and Warfare Agents (Project III)
VA-6D	DNA Damage from Chemical Agents and Its Repair (Project IV)
VA-6E	Clinical and Epidemiology Leishmania Research
VA-7	Desert Storm Reunion Survey
VA-8	Psychological Test Data of Gulf War Veterans Over Time
VA-9	Evaluation of Cognitive Functioning in Persian Gulf War Veterans Reporting War-related Health Problems
VA-10	Memory and Attention in PTSD
VA-11	Neuropsychological Functioning in Veterans
VA-12	Psychological Assessment of Operation Desert Storm Returnees
VA-13	Neurobehavioral Aspects of Persian Gulf Experiences: A Pilot Study
VA-15	Vaccine-Mediated Immunity Against Leishmaniasis
VA-16	Protective Immunity in Experimental Visceral Leishmaniasis
VA-17	Immunological Evaluation of Persian Gulf Veterans
VA-18	Chronic Gastrointestinal Illness in Persian Gulf Veterans

VA-20	Psychological Adjustment in Operation Desert Shield/Storm Veterans
VA-21	A Comparison of PTSD Symptomatology among Three Army Medical Units Involved in ODS
VA-36	Stress Symptoms and Their Causal Attribution in Desert Storm Veterans
VA-40	Musculoskeletal Symptoms in Gulf War Syndrome
VA-46	Diarrhea in Persian Gulf Veterans: An Irritable Bowel-Like Disorder
VA-47	Retrospective Verification of Mustard Gas Exposure
VA-48	Cross-Sensitization as a CNS Model for Gulf War Chemical Intolerance
VA-49	Sensitivity to Pyridostigmine Bromide: Persistent Neural Dysfunction
VA-50	Neuropsychological Findings in a Sample of Operation Desert Storm Veterans
VA-51	Psychobiological Assessment of Desert Storm Veterans
VA-53	Spouses and Children Program
VA-54	Follow-up of Psychological and Neurocognitive Gulf War Outcome: Relation to Stress
VA-55	Antibiotic Treatment of Gulf War Veterans' Illnesses
<u>Department of Defense / Veterans Affairs Shared Projects</u>	
VA/DoD-1D/1V	A Randomized, Multi-Center, Controlled Trial of Multi-Modal Therapy in Veterans with Gulf War Illnesses
VA/DoD-	Follow-Up Investigation of Troops Exposed to Nerve Agents at

2DA/2VA	Aberdeen Proving Ground
VA/DoD-2VB/2DB	Patterns of Pre-Persian Gulf War Illness and Health Care Seeking, Pilot Study
VA/DoD-2D/2V	VA/DoD Core Funding of the Medical Follow-up Agency

Appendix A: project focus areas

Research Type	Secondary Focus Area	Tertiary Focus Area	Project Number	Project Title
Brain & Nervous System				
<i>Mechanistic</i>			<i>DoD-92</i>	Traumatic Experiences <i>Persistently</i> Enhance Cue-dependent Learning: Toward an Animal Model of Chronic Stress and Posttraumatic Stress Disorder
<i>Epidemiology Research</i>	<i>Symptoms/General Health</i>		<i>DoD-23</i>	Acute and Long-Term Impact of Deployment to Southwest Asia on the Physical and Mental Health of Soldiers and their Families
<i>Epidemiology Research</i>	<i>Symptoms/General Health</i>		<i>DoD-52</i>	Female Gender and Other Potential Predictors of Functional Health Status Among Persian Gulf War Veterans
<i>Epidemiology Research</i>			<i>DoD-82</i>	Feasibility of Developing a Registry of PTSD Affected Veteran Sib Pairs
<i>Clinical Research</i>	<i>Prevention</i>		<i>DoD-83</i>	Risk for Stress-related Substance Abuse: the Effects of Family History of Alcoholism
<i>Clinical Research</i>	<i>Symptoms/General Health</i>		<i>DoD-84</i>	Psychobiologic Alterations in Persian Gulf War Veterans with and without PTSD
<i>Clinical Research</i>	<i>Treatment</i>	<i>Symptoms/General Health</i>	<i>DoD-85</i>	CNS Cytokines and CRH in Gulf War Veterans with Multiple Unexplained Symptoms
<i>Clinical Research</i>			<i>DoD-86</i>	Effects of Combat Stress on Structure and Function of the Hippocampus
<i>Epidemiology Research</i>	<i>Prevention</i>		<i>DoD-87</i>	Measurement and Validation of Psychosocial Risk and Resilience

				Mental Health and Health-Related Quality of Life among PGWVs
<i>Clinical Research</i>	<i>Symptoms/General Health</i>		<i>DoD-89</i>	Limbic Blood Flow and Opiate Receptor PET in Posttraumatic Stress Disorder
<i>Clinical Research</i>			<i>VA-20</i>	Psychological Adjustment in Operation Desert Shield/Storm Veterans
<i>Mechanistic</i>			<i>DoD-91</i>	Neurological and Circadian Substrates of PTSD-like Behaviors
<i>Mechanistic</i>			<i>DoD-80</i>	Molecular Regulation of corticosteroid Receptor Expression in stress-Responsive Cells
<i>Clinical Research</i>	<i>Symptoms/General Health</i>		<i>VA-54</i>	Follow-up of Psychological and Neurocognitive Gulf War Outcome: Relation to Stress
<i>Clinical Research</i>			<i>VA-12</i>	Psychological Assessment of Operation Desert Storm Returnees
<i>Clinical Research</i>	<i>Symptoms/General Health</i>		<i>VA-7</i>	Desert Storm Reunion Survey
<i>Clinical Research</i>	<i>Symptoms/General Health</i>		<i>VA-4A</i>	Evaluation of Cognitive Functioning of Persian Gulf Veterans
<i>Clinical Research</i>	<i>Symptoms/General Health</i>		<i>VA-4B</i>	Evaluation of Neurological Functioning in Persian Gulf Veterans
<i>Clinical Research</i>	<i>Symptoms/General Health</i>		<i>VA-10</i>	Memory and Attention in PTSD
<i>Clinical Research</i>	<i>Symptoms/General Health</i>		<i>VA-6A</i>	Psychosocial, Neuropsychological and Neurobehavioral Assessment (Project I)
<i>Clinical Research</i>	<i>Diagnosis</i>		<i>VA-4F</i>	Validity of Computerized Tests
<i>Clinical Research</i>			<i>VA-21</i>	A Comparison of PTSD Symptomatology among Three Army Medical Units Involved in ODS
<i>Clinical Research</i>			<i>VA-13</i>	Neurobehavioral Aspects of Persian Gulf Experiences: A Pilot Study
<i>Clinical Research</i>	<i>Treatment</i>		<i>DoD-90</i>	SPECT Benzodiazepine Receptor and MR Imaging in PTSD
<i>Clinical Research</i>			<i>VA-50</i>	Neuropsychological findings in a sample of Operation Desert Storm veterans

<i>Epidemiology Research</i>			VA-36	Stress Symptoms and Their Causal Attribution in Desert Storm Veterans
<i>Clinical Research</i>	<i>Chemical Weapons</i>		DoD-63	PGW Veterans: Epidemiological and Clinical Evidence for Residual Organophosphate Neurotoxicity
<i>Clinical Research</i>	<i>Symptoms/General Health</i>		DoD-41	Evaluation of Muscle Function in Persian Gulf Veterans
<i>Clinical Research</i>	<i>Symptoms/General Health</i>		DoD-40	Psychological and Neurobiological Consequences of the Gulf War Experience
<i>Clinical Research</i>	<i>Symptoms/General Health</i>		DoD-36	Fatigue in Persian Gulf Syndrome- Physiologic Mechanisms
<i>Clinical Research</i>	<i>Symptoms/General Health</i>	<i>Diagnosis</i>	DoD-67	Antibacterial Treatment method based upon the excretion of dead and decaying spherical bacteria
<i>Clinical Research</i>	<i>Symptoms/General Health</i>		VA-51	Psychobiological Assessment of Desert Storm Veterans
<i>Clinical Research</i>	<i>Diagnosis</i>		DoD-32	Neuropsychological Functioning in Persian Gulf Era Veterans
<i>Clinical Research</i>	<i>Symptoms/General Health</i>	<i>Diagnosis</i>	DoD-31	Dysregulation of the Stress Response in the Persian Gulf Syndrome
<i>Clinical Research</i>	<i>Symptoms/General Health</i>		VA-11	Neuropsychological Functioning in Veterans
<i>Clinical Research</i>	<i>Symptoms/General Health</i>		VA-9	Evaluation of Cognitive Functioning in Persian Gulf War Veterans Reporting War-related Health Problems
<i>Mechanistic</i>	<i>Treatment</i>		DoD-6A	Combat Stress Pharmacotherapy
<i>Clinical Research</i>	<i>Diagnosis</i>	<i>Prevention</i>	DoD-6B	Combat Stress Diagnosis, PTSD Prevention
<i>Clinical Research</i>	<i>Symptoms/General Health</i>		HHS-5	Cognitive Function and Symptom Patterns in Persian Gulf Veterans
Chemical Weapons				
<i>Clinical Research</i>	<i>Diagnosis</i>		VA-47	Retrospective Verification of Mustard Gas Exposure
<i>Mechanistic</i>	<i>Prevention</i>		DoD-51	Transgenic Engineering of Cholinesterases: <i>Tools for Exploring</i> Cholinergic Responses
<i>Mechanistic</i>			DoD-50	Toxicokinetics of O-Ethyl S-(2-

				Methylphosphonothioate [(+)-VX] in Rats, Hairless Guinea Pigs and Marmosets - Identification of Metabolic Pathways
<i>Mechanistic</i>	<i>Brain & Nervous System</i>		<i>DoD-53</i>	Long-Term Effects of Subclinical Exposures to Sarin
<i>Mechanistic</i>	<i>Diagnosis</i>		<i>VA-6D</i>	DNA Damage from Chemical Agents and Its Repair (Project IV)
<i>Epidemiology Research</i>			<i>VA/DoD-2DA**</i>	Follow-Up investigation of troops exposed to nerve agents at Aberdeen Proving Ground
<i>Clinical Research</i>	<i>Pyridostigmine Bromide</i>	<i>Prevention</i>	<i>DoD-60</i>	Butyrylcholinesterase Genetic Variants in Persons with Gulf War Illness
<i>Development</i>	<i>Diagnosis</i>		<i>DoD-49</i>	Diagnosis and Dosimetry of Exposure to Sulfur Mustard: Development of Standard Operating Procedures and Exploratory Research on Protein Adducts
<i>Epidemiology Research</i>	<i>Symptoms/General Health</i>		<i>DoD-69</i>	Five Year Follow-Up of arm Personnel Exposed to Chemical Warfare Agents
Depleted Uranium				
<i>Mechanistic</i>	<i>Environmental Toxicology</i>		<i>DoD-7B</i>	Carcinogenicity of Depleted Uranium Fragments
<i>Mechanistic</i>	<i>Environmental Toxicology</i>		<i>DoD-7A</i>	Health Risk Assessment of Embedded Depleted Uranium: Behavior, Physiology, Histology, and Biokinetic Modeling
Diagnosis				
<i>Clinical Research</i>			<i>DoD-47</i>	Study of Mycoplasmal Infections in Gulf War Veterans
<i>Clinical Research</i>	<i>Symptoms/General Health</i>		<i>HHS-6</i>	Defining Gulf War Illness
<i>Development</i>			<i>DoD-66</i>	Testing for mycoplasmal infection replicability of nucleoprotein gene tracking and forensic polymerase chain reaction
<i>Development</i>			<i>DoD-12</i>	Forward Deployable Diagnostics for Infectious Diseases
Environmental Toxicology				
<i>Mechanistic</i>	<i>Prevention</i>		<i>VA-4E</i>	The Aromatic Hydrocarbon Receptor (AhR) as a Biomarker of Susceptibility
<i>Clinical</i>	<i>Symptoms/General Health</i>		<i>VA-4D</i>	Evaluation of Respiratory Dysfunction Among Gulf War Veterans

<i>Research</i>				
<i>Clinical Research</i>	<i>Brain & Nervous System</i>	<i>Interactions</i>	<i>VA-48</i>	Cross-Sensitization as a CNS Model for Gulf War Chemical Intolerance
<i>Epidemiology Research</i>			<i>VA-4C</i>	Gulf War And Vietnam Veterans Cancer Incidence Surveillance
<i>Development</i>			<i>DoD-16</i>	Kuwait Oil Fire Health Risk Assessment
<i>Development</i>	<i>Interactions</i>		<i>DoD-34</i>	Characterization of Emissions from Heaters Burning Leaded Diesel Fuel in Unvented Tents
<i>Development</i>			<i>DoD-19</i>	Persian Gulf Veterans Health Tracking System
<i>Development</i>			<i>DoD-18</i>	Kuwait Oil Fires Troop Exposure Assessment Model (TEAM)
<i>Epidemiology Research</i>			<i>DoD-13</i>	Effects of Persian Gulf War Service on Military Working Dogs
<i>Mechanistic</i>	<i>Brain & Nervous System</i>	<i>Chemical Weapons</i>	<i>DoD-22</i>	Chronic Organophosphorous Exposure and Cognition
<i>Mechanistic</i>	<i>Prevention</i>		<i>HHS-3</i>	Biomarkers of Susceptibility and Polycyclic Aromatic Hydrocarbon (PAH) Exposure in Urine and blood Cell DNA from U.S. Army Soldiers Exposed to Kuwaiti Oil Well Fires
Immune Function				
<i>Mechanistic</i>	<i>Interactions</i>	<i>Environmental Toxicology</i>	<i>HHS-7</i>	Immunotoxicity of Dermal Permethrin and Cis-Urocanic Acid
<i>Clinical Research</i>	<i>Brain & Nervous System</i>		<i>DoD-88</i>	Clinical Relevance of novel Immunological Markers in PTSD
<i>Clinical Research</i>	<i>Symptoms/General Health</i>		<i>VA-17</i>	Immunological Evaluation of Persian Gulf Veterans
<i>Clinical Research</i>	<i>Symptoms/General Health</i>		<i>VA-6B</i>	Clinical and Neuroendocrine Aspects of Fibromyalgia (Project II)
<i>Clinical Research</i>			<i>DoD-48</i>	Assessment of Genomic Instability via Chromosome 7 Inversion Frequency in a Gulf-War Syndrome Cohort vs Selected Control Groups
<i>Clinical Research</i>	<i>Symptoms/General Health</i>	<i>Environmental Toxicology</i>	<i>VA-5C</i>	Effects of Exertion and Chemical Stress on Persian Gulf Veterans
<i>Clinical Research</i>	<i>Brain & Nervous System</i>		<i>VA-5B</i>	Physiological and Psychological Assessments of Persian Gulf Veterans

Interactions				
<i>Mechanistic</i>	<i>Pyridostigmine Bromide</i>	<i>Brain & Nervous System</i>	<i>DoD-75</i>	Toxic Interactions of Prophylactic Drugs and Pesticides
<i>Mechanistic</i>	<i>Chemical Weapons</i>	<i>Brain & Nervous System</i>	<i>DoD-72</i>	Long-term Effects of Subchronic Exposure to Sarin, Alone and with Stress or Other Chemicals
<i>Mechanistic</i>	<i>Immune Function</i>	<i>Pyridostigmine Bromide</i>	<i>DoD-81</i>	Immunotoxicity due to Coexposure to DEET, Pyridostigmine, and Stress
<i>Mechanistic</i>	<i>Chemical Weapons</i>	<i>Brain & Nervous System</i>	<i>DoD-78</i>	Experimental Models of Gulf War Syndrome
<i>Mechanistic</i>	<i>Pyridostigmine Bromide</i>	<i>Brain & Nervous System</i>	<i>DoD-2</i>	Physiological and Neurobehavioral Effects in Rodents from Exposure to Pyridostigmine, Fuels, and DEET
<i>Mechanistic</i>	<i>Pyridostigmine Bromide</i>	<i>Immune Function</i>	<i>DoD-76</i>	Evaluations of Immunotoxicity due to Concurrent Exposure to DEET, Pyridostigmine, and JP-8 Jet Fuel
<i>Mechanistic</i>	<i>Chemical Weapons</i>	<i>Pyridostigmine Bromide</i>	<i>DoD-62</i>	Sarin and Pyridostigmine Interaction under Physical Stress: Neurotoxic Effects in Mice
<i>Development</i>	<i>Environmental Toxicology</i>		<i>HHS-8</i>	Strategy to Identify Non-Additive Response to Chemical Mixtures
<i>Mechanistic</i>	<i>Pyridostigmine Bromide</i>	<i>Brain & Nervous System</i>	<i>DoD-37</i>	Neurobehavioral and Immunological Toxicity of Pyridostigmine, Permethrin, and DEET in Male and Female Rats
<i>Mechanistic</i>	<i>Pyridostigmine Bromide</i>		<i>DoD-77</i>	Percutaneous Absorption of Chemical Mixtures Relevant to the Gulf War
<i>Mechanistic</i>	<i>Chemical Weapons</i>	<i>Pyridostigmine Bromide</i>	<i>DoD-61</i>	Neurophysiologic and Neuropathologic Effects in Monkeys of Low Level Exposures to Sarin, Pyridostigmine, Pesticides, and Botulinum Toxoid
<i>Mechanistic</i>	<i>Chemical Weapons</i>	<i>Pyridostigmine Bromide</i>	<i>DoD-56</i>	Low-Level Sarin Neurotoxicity and Its Modulation by Pyridostigmine
<i>Mechanistic</i>	<i>Brain & Nervous System</i>	<i>Chemical Weapons</i>	<i>DoD-54</i>	Assessment of Subchronic Neurobehavioral and Neuropathologic Effects in Rats
<i>Mechanistic</i>	<i>Chemical Weapons</i>	<i>Pyridostigmine Bromide</i>	<i>DoD-55</i>	Low-Level Exposure to GB Vapor in Air: Diagnosis/Dosimetry, Lowest Observable Effect Levels, Performance-Incapacitation, and Possible Delayed Effects
Leishmaniasis				
<i>Mechanistic</i>	<i>Prevention</i>		<i>VA-15</i>	Vaccine-Mediated Immunity Against Leishmaniasis

<i>Development</i>	<i>Diagnosis</i>		<i>DoD-8B</i>	Development of a Leishmania Skin Test Antigen (LSTA)
<i>Clinical Research</i>	<i>Diagnosis</i>	<i>Treatment</i>	<i>VA-6E</i>	Clinical and Epidemiology Leishmania <i>Research</i>
<i>Mechanistic</i>	<i>Treatment</i>		<i>DoD-9</i>	Identification of the Genetic Factors Which Control Tropism in Leishmania
<i>Mechanistic</i>	<i>Prevention</i>		<i>VA-16</i>	Protective Immunity in Experimental Visceral Leishmaniasis
<i>Development</i>	<i>Diagnosis</i>		<i>DoD-38</i>	Diagnostic Antigens of Leishmania tropica
<i>Development</i>	<i>Diagnosis</i>		<i>DoD-8A</i>	Serologic Diagnosis of Viscerotropic Leishmaniasis (VTL)
Mortality				
<i>Epidemiology Research</i>			<i>VA-1</i>	Mortality Follow-up Study of Persian Gulf Veterans, First Update
<i>Epidemiology Research</i>			<i>DoD-15</i>	Comparative Mortality Among US Military Personnel Worldwide During Operations Desert Shield and Desert Storm
Pyridostigmine Bromide				
<i>Mechanistic</i>	<i>Environmental Toxicology</i>		<i>VA-6C</i>	Neurotoxicity of Environmental Pollutants and Warfare Agents (Project III)
<i>Epidemiology Research</i>			<i>DoD-17</i>	Retrospective Studies Involving Military Use of Pyridostigmine as a Pretreatment for Nerve Agent Poisoning
<i>Clinical Research</i>	<i>Brain & Nervous System</i>	<i>Prevention</i>	<i>DoD-64</i>	Individual Differences in Neurobehavioral Effects of Pyridostigmine
<i>Mechanistic</i>	<i>Prevention</i>		<i>DoD-33</i>	Effects of Pyridostigmine in Flinders Line Rats Differing in Cholinergic Sensitivity
<i>Mechanistic</i>	<i>Interactions</i>		<i>DoD-10</i>	Pyridostigmine Synergistic Toxicity Study
<i>Clinical Research</i>			<i>DoD-11</i>	Male/Female Differential Tolerances to Pyridostigmine Bromide
<i>Epidemiology Research</i>	<i>Prevention</i>		<i>DoD-21</i>	Study of Variability In Pyridostigmine Inhibition of Blood Cholinesterases in Healthy Adults and Individuals With

				Operation Desert Storm
<i>Mechanistic</i>	<i>Brain & Nervous System</i>		<i>DoD-59</i>	<i>Pyridostigmine-induced Neurodegeneration: Role of neuronal Apoptosis</i>
<i>Mechanistic</i>	<i>Brain & Nervous System</i>		<i>DoD-79</i>	Time Course of Stress-induced Impairment of Blood Brain Barrier
<i>Mechanistic</i>	<i>Brain & Nervous System</i>		<i>VA-49</i>	Sensitivity to Pyridostigmine Bromide: Persistent Neural Dysfunction
<i>Mechanistic</i>	<i>Interactions</i>		<i>VA-5D</i>	Effects of Genetics and Stress on Responses to Environmental Toxins
Reproductive Health				
<i>Epidemiology Research</i>			<i>HHS-4</i>	Suspected Increase of Birth Defects and health Problems Among Children Born to Persian Gulf War Veterans in Mississippi
<i>Epidemiology Research</i>			<i>DoD-1C</i>	Epidemiologic Studies of Morbidity Among Gulf War Veterans: A Search for Etiologic Agents and Risk Factors; Study 3: A comparative study of pregnancy outcomes among Gulf War veterans and other active-duty personnel
<i>Epidemiology Research</i>			<i>DoD-1G</i>	Epidemiologic Studies of Morbidity Among Gulf War Veterans: A Search for Etiologic Agents and Risk Factors; Study 7: Prevalence of Congenital Anomalies Among Children of Persian Gulf War Veterans
<i>Epidemiology Research</i>			<i>DoD-1D</i>	Epidemiologic Studies of Morbidity Among Gulf War Veterans: A Search for Etiologic Agents and Risk Factors; Study 4: Adverse Reproductive Outcomes in Gulf War Veterans
<i>Epidemiology Research</i>			<i>DoD-35</i>	Feasibility of Investigating Whether There is a Relationship Between Birth Defects and Service in the Gulf War.
<i>Clinical Research</i>	<i>Symptoms/General Health</i>	<i>Immunology</i>	<i>DoD-44</i>	Investigation of Seminal Plasma Hypersensitivity Reactions
Symptoms/General Health				
<i>Epidemiology Research</i>	<i>Diagnosis</i>		<i>HHS-2</i>	Disease Cluster in a Pennsylvania Air National Guard Unit, EPI-AID 95-18
<i>Epidemiology Research</i>	<i>Reproductive Health</i>		<i>DoD-30</i>	Epidemiological Studies Persian Gulf War Illnesses, PG Women's Health Linkage Study
<i>Epidemiology Research</i>			<i>DoD-46</i>	Exploratory Data Analysis with the CCEP Database

<i>Clinical Research</i>	<i>Brain & Nervous System</i>		<i>DoD-58</i>	Illness Among Persian Gulf War Veterans: Case Validation Studies
<i>Clinical Research</i>	<i>Brain & Nervous System</i>		<i>DoD-57</i>	Physiologic Effects of Stress in Gulf War Veterans
<i>Clinical Research</i>	<i>Immunology</i>	<i>Brain & Nervous System</i>	<i>DoD-42</i>	The Symptomatic Persian Gulf Veterans Protocol: An Analysis of Risk Factors with an Immunologic and Neuropsychiatric Assessment
<i>Clinical Research</i>	<i>Brain & Nervous System</i>	<i>Reproductive Health</i>	<i>DoD-45</i>	Physical and Emotional Health of Gulf War Veterans Women
<i>Clinical Research</i>	<i>Brain & Nervous System</i>		<i>VA-4Core</i>	Boston Environmental Hazards Research Center Program
<i>Clinical Research</i>			<i>VA-40</i>	Musculoskeletal Symptoms in Gulf War Syndrome
<i>Epidemiology Research</i>			<i>VA-46</i>	Diarrhea in Persian Gulf Veterans: An Irritable Bowel-Like Disorder
<i>Epidemiology Research</i>			<i>DoD-39</i>	A Controlled Epidemiological and Clinical Study into the Effect of Gulf War Service on Servicemen and Women of the United Kingdom Armed Forces
<i>Epidemiology Research</i>			<i>DoD-74</i>	Relationship of Stress Exposures to Health in Gulf War Veterans
<i>Clinical Research</i>	<i>Brain & Nervous System</i>		<i>VA-8</i>	Psychological Test Data of Gulf War Veterans Over Time
<i>Epidemiology Research</i>			<i>VA-5A</i>	Health and Exposure Survey of Persian Gulf Veterans
<i>Epidemiology Research</i>			<i>VA-6Core</i>	Core Program: Portland Environmental Hazards Research Center: Environment, Veterans Health and the Gulf War Syndrome.
<i>Epidemiology Research</i>			<i>HHS-1</i>	Health Assessment of Persian Gulf War Veterans from Iowa
<i>Clinical Research</i>			<i>DoD-1A</i>	Epidemiologic Studies of Morbidity Among Gulf War Veterans: A Search for Etiologic Agents and Risk Factors; Study 1: A Study of Symptoms Among 1500 Seabees
<i>Epidemiology Research</i>			<i>DoD-1B</i>	Epidemiologic Studies of Morbidity Among Gulf War Veterans: A Search for Etiologic Agents and Risk Factors; Study 2: A comparative study of hospitalizations among Active-duty personnel who participated in the Gulf War and similar personnel who did not.

<i>Clinical Research</i>	<i>Brain & Nervous System</i>		<i>VA-5Core</i>	East Orange Environmental Hazards Research Center Program
<i>Epidemiology Research</i>			<i>DoD-1E</i>	Epidemiologic Studies of Morbidity Among Gulf War Veterans: A Search for Etiologic Agents and Risk Factors; Study 5: Seabee Health Study.
<i>Epidemiology Research</i>			<i>DoD-1F</i>	Epidemiologic Studies of Morbidity Among Gulf War Veterans: A Search for Etiologic Agents and Risk Factors; Study6: A Comparison of Nonfederal Hospitalization Experience Among Veterans in California who have separated from active service: PGW vs EV.
<i>Epidemiology Research</i>			<i>DoD-14</i>	Risk Factors Among US Army Soldiers for Enrolling on the Department of Veterans Affairs Gulf War Registry
<i>Epidemiology Research</i>			<i>DoD-71</i>	A Comparison of Post Deployment Hospitalization Between Vietnam and Gulf War Veterans
<i>Epidemiology Research</i>			<i>DoD-4</i>	The General Well-Being of Gulf War Era Service Personnel from the States of Pennsylvania and Hawaii: A Survey
<i>Epidemiology Research</i>			<i>VA/DoD-2DB**</i>	Patterns of Pre-Persian Gulf War Illness and Health Care Seeking, Pilot Study
<i>Epidemiology Research</i>			<i>VA-2</i>	National Health Survey of Persian Gulf Veterans
<i>Epidemiology Research</i>			<i>DoD-73</i>	Post-deployment Morbid Stress, Behavior and Health: Developing a Model for Predicting Morbidity, Mortality, and other Adverse Outcomes
<i>Clinical Research</i>			<i>VA-18</i>	Chronic Gastrointestinal Illness in Persian Gulf Veterans
<i>Epidemiology Research</i>			<i>DoD-70</i>	War Syndromes from 1900 to the Present: Symptom Patterns and Long-term Health Outcomes
<i>Epidemiology Research</i>			<i>VA-2A</i>	VA National Survey of Persian Gulf Veterans - Phase I
<i>Epidemiology Research</i>			<i>VA-2B</i>	VA National Survey of Persian Gulf Veterans - Phase II
<i>Epidemiology Research</i>	<i>Brain & Nervous System</i>	<i>Diagnosis</i>	<i>VA-2C</i>	VA National Survey of Persian Gulf Veterans - Phase III
<i>Epidemiology Research</i>			<i>VA-3</i>	Use of Roster of Veterans Who Served in Persian Gulf Area

<i>Epidemiology Research</i>			VA/DoD-2VB**	Patterns of Pre-Persian Gulf War Illness and Health Care Seeking, Pilot Study
<i>Epidemiology Research</i>			DoD-20	A Statistical Study Correlating the Reported Cases of Gulf War Syndrome to Battlefield Locations of Afflicted US Army Personnel During the Iraq-Kuwait War; Part 1: Method to Related Troop Deployment and the Reported Cases of Gulf War Syndrome and Probable Incidence of Maladies Defined by ICD-9-CM
Treatment				
<i>Clinical Research</i>	<i>General Health</i>		VA-55	Antibiotic Treatment of Gulf War Veterans' Illnesses
<i>Clinical Research</i>			DoD-65	Multi-disciplinary Pathophysiologic studies of neurotoxic Gulf War related syndromes leading to diagnosis and treatment
<i>Clinical Research</i>	<i>Symptoms/General Health</i>		VA/DoD-1D**	A Randomized, Multi-Center, Controlled Trial of Multi-Modal Therapy in Veterans with Gulf War Illnesses
<i>Clinical Research</i>	<i>Symptoms/General Health</i>		VA/DoD-1V**	A Randomized, Multi-Center, Controlled Trial of Multi-Modal Therapy in Veterans with Gulf War Illnesses

Appendix A: project abstracts

Title: Naval Health Study Program
Project #: DoD-1
Agency: DoD
Study Location: Naval Health Research Center
Project Status: Ongoing
P.I.: CAPT Greg Gray, MC. USN
 This is the parent Program for DoD projects 1A through 1G.
PUBLICATIONS: See DoD-1

Title: Epidemiologic Studies of Morbidity Among Gulf War Veterans: A Search for Etiologic Agents and Risk Factors; Study 1: A Study of Symptoms Among 1500 Seabees
Project #: DoD-1A
Agency: DoD
Study Location: Naval Health Research Center

Project Status: Complete

Research Type: Clinical Research

P.I.: CAPT Greg Gray, MC, USN

Research Focus: Symptoms/General Health

Start Date (CY): 1994

Est. Completion (CY): 1997

OVERALL PROJECT OBJECTIVE: To identify risk factors associated with reported symptoms in an effort to determine causes for possible Gulf War-related morbidity.

SPECIFIC AIMS: Are there differences in postwar morbidity between Gulf War veterans (GWV) and nondeployed veterans (NDV)?

METHODOLOGY: Seabees who were on active duty and had been so since September 1990 were eligible to participate. Seabees (Navy Construction Battalion Workers) were interviewed at two major Seabee bases, in Port Hueneme, CA, and Gulfport, MS. After signing a consent form, volunteers completed a questionnaire, provided blood and urine specimens, and had their height, weight, and handgrip strength measured. A systematically selected subsample also performed a spirometry test. Whole blood, sera, and urine specimens were stored at -70oC. Sera were studied for evidence of infections while whole blood specimens may be characterized for genetic markers that may explain symptoms. Sera collected during this study were compared to prewar sera. Urine specimens may be used to rule out chronic diseases, such as adrenal insufficiency. Handgrip strength and spirometry results were compared among symptomatic and nonsymptomatic Seabees. The questionnaire responses were used to compare the morbidity of GWV and NDV. Internal comparisons were made among GWV using logistic regression modeling to determine if any specific exposures are associated with any symptoms or symptom complexes.

EXPECTED PRODUCTS (MILESTONES): A comparison of morbidity between GWV and NDV Seabees was performed. Survey screening tools was used to detect post traumatic stress disorder, chronic fatigue syndrome and three diagnostic symptom patterns. Assays were performed on pre and postwar sera as to detect infections from Mycoplasma fermentans. Sera were also tested for nonspecific reactants: C-reactive protein, transferrin, ferritin and haptoglobin. Three spin-off round review the postwar strength.

STATUS/RESULTS TO DATE: Seabees (n=1498) were surveyed and studied. Deployed Seabees self-reported a higher prevalence of exposures and symptoms, as well as higher scores for abnormal psychological variables. GWVs were more likely to screen for post traumatic stress disorder, had lower handgrip strengths, and higher serum ferritin assays, however, after numerous comparisons of these outcomes with various exposures, no exposure stood out as being etiologic. We have conducted more in-depth analyses of symptom data using factor analysis. A number of manuscripts and abstracts have been developed.

PUBLICATIONS: Gray GC, Kaiser KS, Hawksworth AW, Watson HL. No serological evidence of an association found between Gulf War service and Mycoplasma fermentans infection. Am J Trop Med Hyg, 1998; in press.

Knoke JD, Smith TC, Gray GC, Kaiser KS, Hawksworth AW. Factor analysis: Does it identify a Gulf War syndrome? Under external review.

Kaiser KS, Hawksworth AW, Gray GC. Pyridostigmine bromide intake during the Persian Gulf War not associated with postwar handgrip strength. Under external review.

Gray GC, Kaiser KS, Hawksworth AW, Hall FW, Barrett-Connor EL. Increased postwar symptoms and psychological morbidity among US Navy Gulf War veterans. Under external review.

McDonough C, Gray GC. Topical bibliography of published works regarding the health of veterans of the Persian Gulf War. Technical Document No. 95-4. Revised in Smith, T. The capabilities of proc factor to conduct an exploratory factor analysis. Proceedings for the Western Users of SAS Software, 6th Annual Conference, October 14-15, 1998, Oakland, California.

Knoke JD, Smith TC, Gray GC, Kaiser KS, Hawksworth AW. Factor analysis of self-reported symptoms. Does it identify a Gulf War syndrome? Conference on Federally Sponsored GWVs'

Title: Epidemiologic Studies of Morbidity Among Gulf War Veterans: A Search for Etiologic Agents and Risk Factors; Study 2: A Comparative Study of Hospitalizations among Active-duty Personnel Who Participated in the Gulf War and Similar Personnel Who Did Not

Project #: DoD-1B

Agency: DoD

Study Location: Naval Health Research Center

Project Status: Complete

Research Type: Epidemiology Research

P.I.: CAPT Greg Gray, MC, USN

Research Focus: Symptoms/General Health

Start Date (CY): 1994

Est. Completion (CY): 1998

OVERALL PROJECT OBJECTIVE: Screen Department of Defense (DoD) hospitalizations for Association with Gulf War deployment; identify specific diseases or disease groups that merit further study.

SPECIFIC AIMS: Are hospitalization rates the same for Gulf War veterans (GWV) and nondeployed veterans (NDV)?

EXPECTED PRODUCTS (MILESTONES): These data have been proven useful beyond their original intent in examining new hypothesis regarding specific Gulf War exposure and specific hospitalization outcomes. A number of spin-off studies have been published in leading journals (4), are in internal review (1), are in external review for publication (2), or are in progress (1). In one study we are examining the postwar hospitalization experience of personnel possibly exposed to Iraqi chemical munitions destruction (in external review). In another study, we examined the postwar hospitalization experience of Gulf War Veterans for a group of diagnoses most likely to capture an emerging illness (published). Additionally, we are conducting further analyses of the mental illness hospitalizations and other more specific diagnosis (in external review).

STATUS/RESULTS TO DATE: In general, Gulf War veterans postwar hospitalization data are very similar to that of their nondeployed peers. Thus far, there has been little evidence on unexplained increased postwar hospitalization morbidity associated with Gulf War service. A number of abstracts and manuscripts have been published or are under external review for publication.

PUBLICATIONS: Knoke JD, Gray GC, Garland FC. Lack of association of testicular cancer with Persian Gulf War service. *Epidemiology*, 9:648-653, 1998.

Gray GC, Hawksworth AW, Smith TC, Kang HK, Knoke JD, Gackstetter GD. Gulf War veterans' health registries. Who is most likely to seek evaluation? *Am J Epidemiol*, 148:343-349, 1998.

Gray GC, Knoke JD, Berg SW, Wignall FS, Barrett-Connor E. Counterpoint: Responding to suppositions and misunderstandings. *Am J Epidemiol*, 148:328-333, 1998.

Knoke JD, Gray GC. Hospitalizations for unexplained illnesses among US Persian Gulf War veterans. *Emerging Infectious Diseases*, 4:211-218, 1998.

Knoke JD, Gray GC. Hospitalizations after the Gulf War - Reply to K.M. Leisure, et al. *Emerging Infectious Diseases*. 4:709, 1998, Letter.

Gray GC, Coate BD, Anderson CM, Kang HK, Berg SW, Wignall FS, Knoke JD, Barrett-Connor E. The postwar hospitalization experience of US Persian Gulf War veterans. *N Engl J Med*, 335:1505-13, 1996.

Gray GC, Smith TC, Knoke JD, Heller JM. Hospitalization risk after possible exposure to Iraqi chemical munitions destruction during the Persian Gulf War. Conference on Federally Sponsored Gulf War Veterans' Illnesses Research. June 17-19. 1998. Arlington. Virginia.

Knoke JD, Smith TC, Gray GC, Kaiser KS, Hawksworth AW. Lack of association between testicular cancer and Persian Gulf war service. Conference on Federally Sponsored Gulf War Veterans' Illnesses Research, June 17-19, 1998, Arlington, VA.

Smith TC, Gray GC, Knoke JD. Hospitalization risk of Gulf War veterans for Lupus Erythematosus, Amyotrophic Lateral Sclerosis, and Fibromyalgia. Conference on Federally Sponsored Gulf War Veterans' Illnesses Research, June 17-19, 1998, Arlington, Virginia.

Reed RJ, Knoke JD, Gray GC. Hospitalization for alcohol-related disorders in the US Armed Forces: 1 June 1991-30 September 1993. 39th Annual Navy Occupational and Preventive Medicine Workshop, March 28-April 3, 1998, San Diego, California

Title: Epidemiologic Studies of Morbidity Among Gulf War Veterans: A Search for Etiologic Agents and Risk Factors; Study 3: A comparative study of pregnancy outcomes among Gulf War veterans and other active-duty personnel

Project #: DoD-1C

Agency: DoD

Study Location: Naval Health Research Center

Project Status: Complete

Research Type: Epidemiology Research

P.I.: CAPT Greg Gray, MC, USN

Research Focus: Reproductive Health

Start Date (CY): 1994

Est. Completion (CY): 1997

OVERALL PROJECT OBJECTIVE: To evaluate the risk of birth defects and other adverse pregnancy outcomes diagnosed in military medical treatment facilities (MTFs). Risk and relative risk of inpatient events will be assessed based on existing data.

SPECIFIC AIMS: Within the design parameters, are Persian Gulf War veterans (GWV) at higher risk of adverse pregnancy outcomes than nondeployed veterans (NDV)?

METHODOLOGY: This was an historical cohort study of reproductive outcomes among military personnel deployed to the Gulf War compared to military personnel who were not deployed. Demographic and hospitalization data were available for approximately 500,000 GWV and 700,000 NDV from the Department of Defense Manpower Data Center. Substantial demographic information was available on all subjects, including race/ethnicity, proxy measures of socio-economic status (rank and education), and military occupation. Data on inpatient services received from MTFs were also available and diagnoses were recorded using ICD-9-CM codes. Risk and relative risks of major birth defects were evaluated. Potentially confounding factors such as maternal age were evaluated. Multivariate analysis was conducted to estimate relative risks, controlling for identified cofounders. Events among female service members were considered separately from those among spouses of male service members. In another study we measured the prevalence of Goldenhar syndrome among infants born in military hospitals to GWV and NDV. This project is now completed.

EXPECTED PRODUCTS (MILESTONES): A number of manuscripts and abstracts have been generated. These data may be used to test additional hypotheses regarding specific subgroups of Gulf War veterans and specific birth defect diagnoses.

STATUS/RESULTS TO DATE: See Publications.

PUBLICATIONS: Cowan DN, Gray GC, DeFrait RF. Counterpoint: Responding to inadequate critique of birth defects paper. *Amer J Epidemiol*, 148:326-327, 1998.

Araneta MRG, Moore CA, Olney RS, Edmonds LD, Karcher JA, McDonough C, Hiliopoulos KM, Schlangen KM, Gray GC. Goldenhar syndrome among infants born in military hospitals to Gulf War veterans. *Teratology*, 56:244-251, 1997.

Cowan DN, DeFrait RF, Gray GC, Goldenbaum M, Wishik S. The risk of birth defects among children of Persian Gulf War veterans. *N Engl J Med*. 336:1650-56. 1997.

Cowan DN, DeFraités RF, Gray GC, Goldenbaum M. Birth defects among children of Gulf War veterans. *Amer J Epidemiol*, 145:S12, 1997, Abstract.
Cowan DN, Gray GC, DeFraités RF. The authors reply to 'Birth defects among children of Persian Gulf War veterans.' *N Engl J Med*, 337:1175-6, 1997, Letter.
Gastanaga VM, Araneta MRG, Hiliopoulos KM, Kamens DR, Schlangen KM, Zau AC. Endogenous predictors in multiple regression: Birthweight and gestational age among Gulf War-exposed pregnancies. Conference on Federally Sponsored Gulf War Veterans' Illness Research, June 17-19, 1998, Arlington, Virginia.
Araneta MRG, Kamens DR, Gastanaga VM, Zau AC, Schlangen KM, Hiliopoulos KM, Gray GC. Reproductive and perinatal outcomes among conceptions and pregnancies during the Persian Gulf War. Conference on Federally Sponsored Gulf War Veterans' Illness Research, June 17-19, 1998, Arlington, Virginia.
Kamens DR, Gastanaga VM, Hiliopoulos KM, Schlangen KM, Zau AC, Araneta MRG. The Gulf War pregnancies study - A comparison of response rates. 1998 San Diego Biostatistics and Epidemiology Research Exchange, La Jolla, California

Title: Epidemiologic Studies of Morbidity Among Gulf War Veterans: A Search for Etiologic Agents and Risk Factors; Study 4: Adverse Reproductive Outcomes in Gulf War Veterans

Project #: DoD-1D

Agency: DoD

Study Location: Naval Health Research Center

Project Status: Ongoing

Research Type: Epidemiology Research

P.I.: CAPT Greg Gray, MC, USN

Research Focus: Reproductive Health

Start Date (CY): 1994

Est. Completion (CY): 1999

OVERALL PROJECT OBJECTIVE: To examine if there are differences between Gulf War veterans (GWVs) and nondeployed veterans (NDVs) in rates of adverse reproductive outcomes.

SPECIFIC AIMS: Do GWVs have higher rates of adverse reproductive outcomes such as miscarriages, early pregnancy loss, prematurity and low birth weight when compared to NDVs?

METHODOLOGY: A structured, self-completed, questionnaire was mailed to 17,166 service members (active duty, reserves, or National Guard) who were, on February 1, 1991, aged between 18-33 and either: married at that time; or currently married. Detailed demographic, military and deployment status, and reproductive outcome data through the end of 1995 were collected for the subject and their marital partner.

EXPECTED PRODUCTS (MILESTONES): Mailing of questionnaires completed (Fall, 1997); data cleanup and telephone callback interviews completed (Spring, 1998). Final cleanup and preparation for data analysis in process (target completion: January, 1999). In another study, we are examining the characterizations of adverse reproductive and perinatal effects among conceptions, which occurred to the Persian Gulf War.

STATUS/RESULTS TO DATE: Initial mailing to the randomly selected GWV and NDV was completed over summer, 1996; two follow-up mailings to increase the participation rate were completed in winter, 1996 and summer, 1997 respectively. Despite 3 attempts, use of locator services, and certified mail or military channels for non-active duty and active duty subjects respectively, questionnaires were undeliverable to 2,503 of the initially selected subjects. At the completion of the mailing phase, a total of 9,691 questionnaires had been returned, giving an overall participation rate of 66.1%. Preliminary analysis of data (Summer, 1998) suggested only that deployed female veterans had fewer births in 1991, consistent with their deployment status, and Department of Defense policies of non-deployment of service members who are known to be pregnant.

PUBLICATIONS: Sato PA, Hiliopoulos KM, Gray GC, Barbour JD, Kamens DR, Morn CD, Poblete PP, Zau AC. The Persian Gulf War - Effects on human reproduction. 39th Annual Navy Occupational and Preventive Medicine Workshop, March 28-April 3, 1998, San Diego, California.

Kamens DR, Sato PA, Morn CB, Poblete PP, Zau AC, Gray GC. The reproductive outcomes study - A comparison of responses within exposure groups. 39th Annual Navy Occupational and Preventive Medicine Workshop, March 28-April 3, 1998, San Diego, California.

Hiliopoulos KM, Araneta MRG, Destiche DA, Sato PA, Barbour JA, Zau AC, Gray GC. The Persian Gulf War: Comparison of perinatal hospitalization data. 39th Annual Navy Occupational and Preventive Medicine Workshop, March 28-April 3, 1998, San Diego, California.

Sato PA, Hiliopoulos KM, Gray GC, Kamens DR, Morn CB, Poblete PP, Zau AC. The national study on reproductive outcomes. Initial findings. Conference on Federally Sponsored Gulf War Veterans' Illnesses Research, June 17-19, 1998, Arlington, VA.

Zau AC, Sato PA, Hiliopoulos KM, Kamens DR, Morn CB, Poblete PP, Gray GC. The national study on reproductive outcomes. A demographic comparison of survey types. Conference on Federally Sponsored Gulf War Veterans' Illnesses Research, June 17-19, 1998, Arlington, Virginia.

Poblete PP, Araneta MRG, Sato PA, Hiliopoulos KM, Kamens DR, Morn CB, Zau AC, Gray GC. National study on reproductive outcomes. A reliability study of self-administered survey versus telephone interview. Conference on Federally Sponsored Gulf War Veterans' Illnesses Research, June 17-19, 1998, Arlington, VA.

Title: Epidemiologic Studies of Morbidity Among Gulf War Veterans: A Search for Etiologic Agents and Risk Factors; Study 5: Seabee Health Study

Project #: DoD-1E

Agency: DoD

Study Location: Naval Health Research Center

Project Status: Ongoing

Research Type: Epidemiology Research

P.I.: CAPT Greg Gray, MC, USN

Research Focus: Symptoms/General Health

Start Date (CY): 1996

Est. Completion (CY): 2013

OVERALL PROJECT OBJECTIVE: To study the health of Navy Seabees (Navy Construction Battalion members) in relation to their deployments and the possible latent effects of Gulf War service.

SPECIFIC AIMS: 1. To determine if Gulf War Veterans have greater frequency of symptoms, illness, and propensity for making cognitive errors than other veterans; 2. to assemble a cohort for research on long-term effects of the Gulf War on morbidity.

METHODOLOGY: The proposed study is a nonconcurrent historical prospective study of all Seabees who were on active duty for at least one month between August 2, 1990 and June 30, 1991. This group of approximately 19,000 current active duty, reservists and former military personnel was identified from records of the Defense Manpower Data Center. The study subjects were mailed a survey to assess their current health status and their role (if any) in the Persian Gulf War. Participants in the survey will be followed prospectively for 15 years with a follow-up survey every 5 years (2003, 2008, 2013) to detect possible latent effects of the Gulf War on health. Potential sequelae include heart disease, diabetes, hypertension and arthritis. To evaluate reliability of information, 400 of the survey participants will be interviewed by telephone and information on health and symptomatology from routine physicals will be collected from military medical records. Bivariate and multivariate analyses will be used to identify risk factors associated with the outcomes of interest and multivariate logistic regression modeling will be applied to evaluate relationships between risk factors and the health outcomes. Development of latent

chronic disease will be monitored by calculating the cumulative prevalence rates after each 5-year survey follow-up and comparing to the baseline prevalence.

EXPECTED PRODUCTS (MILESTONES): Data collection should be completed by March 1999. Subsequent analyses of symptoms and morbidity data from written and telephone survey are expected by March 2003, March 2008, and March 2013. A comprehensive report showing comparisons between Gulf War Veterans and other veterans including a summary of risk factor associations with morbidity will be completed by October 2013.

STATUS/RESULTS TO DATE: Participation is currently at 60% after the third mailing of the survey. Two more mailings are planned to give all subjects an equal opportunity to participate. A reliability study has been completed and a nonrespondent study is being implemented.

PUBLICATIONS: Dlugosz LJ, Gray GC, Knoke J, Kang HK, Larson G. A prospective assessment of symptoms and illness in Gulf War veterans: the Seabee Health Study. 1996 San Diego Statistics and Epidemiology Research Exchange, La Jolla, California.
Reed R, Kaiser KS, Gray GC. The Seabee health Study: results and status after nine months of data collection. Conference on Federally Sponsored Gulf War Veterans' Illnesses Research, Arlington, VA, 1998.

Title: Epidemiologic Studies of Morbidity Among Gulf War Veterans: A Search for Etiologic Agents and Risk Factors; Study 6: A Comparison of Nonfederal Hospitalization Experience Among Veterans in California who have separated from active service: PGW vs. EV

Project #: DoD-1F

Agency: DoD

Study Location: Naval Health Research Center

Project Status: Complete

Research Type: Epidemiology Research

P.I.: CAPT Gregory Gray, MC, USN

Research Focus: Symptoms/General Health

Start Date (CY): 1995

Est. Completion (CY): 1998

OVERALL PROJECT OBJECTIVE: To determine if the federal and non-federal hospitalizations rates differed among Gulf War Veterans (GWV) and nondeployed veterans (NDV) during the study period of 1 August 1991 to 31 December 1994.

SPECIFIC AIMS: This research addressed these questions: (1) Did the causes for hospitalization rates of GWV and NDV differ in (1) military and (2) nonfederal facilities? (2) Did the proportional distribution of causes for hospitalization differ during the postwar follow-up period? (3) Did morbidity patterns differ between GWV and NDV, temporally, and by type of hospital (federal vs. nonfederal)?

METHODOLOGY: The study population consisted of GWV and NDV. Their hospitalizations in Department of Defense, Department of Veterans Affairs and nonfederal California hospitals were compared for several years after the war. Adjusted proportional distributions of hospital diagnoses were compared.

EXPECTED PRODUCTS (MILESTONES): Completed study.

STATUS/RESULTS TO DATE: A computerized linking program was designed by the California OSHPD to enable linkage of their database of nonfederal hospital discharge information to a NHRC database consisting of identifying demographic data for GWV and NDV. Negotiations for the terms of this collaboration were completed and data provided to OSHPD in Spring 1997. Data were linked and returned to NHRC in late November 1997. The data provided to the VA in Spring 1997 was linked and returned to NHRC in July 1997. Manuscript under medical journal review.

PUBLICATIONS: none to

Title: Epidemiologic Studies of Morbidity Among Gulf War Veterans: A Search for Etiologic Agents and Risk Factors; Study 7: Prevalence of Congenital Anomalies Among Children of Persian Gulf War Veterans

Project #: DoD-1G

Agency: DoD

Study Location: Naval Health Research Center

Project Status: Ongoing

Research Type: Epidemiology Research

P.I.: CAPT Gregory Gray, MC, USN

Research Focus: Reproductive Health

Start Date (CY): 1995

Est. Completion (CY): 1999

OVERALL PROJECT OBJECTIVE: To determine if the prevalence of congenital anomalies in the infants born to Gulf War Veterans (GWV) exceed those of infants of (1) nondeployed veterans (NDV) and (2) the nonmilitary population.

SPECIFIC AIMS: The specific research questions this project will address include: (1) Did the prevalence of congenital anomalies differ among infants of GWV, NDV, and the nonmilitary population prior to and after the Persian Gulf War? (2) Did the types of congenital anomalies differ among infants of GWV, NDV, and the nonmilitary population prior to and following the Persian Gulf War? (3) What were the pathologic patterns of congenital anomalies among infants born to GWV? How did these characteristics differ by military subpopulations (e.g., service, occupation, geographic location)?

METHODOLOGY: The Department of Defense Manpower Data Center (DMDC) maintains a database of demographic, military and hospitalization data among military personnel. The state health departments of Arizona, Arkansas, California, Georgia, Hawaii and Iowa conduct active surveillance of congenital anomalies through the initial 12 months of life. Live births and fetal deaths occurring after 20 weeks of gestation are recorded in Birth and Death Certificate Records of the Vital Statistics Registries of these states.

Two registry matches will be performed. The DMDC database will be matched with Vital Statistics Registry of each of these six states to identify live births and fetal deaths occurring among conceptions of GWV and NDV between 1989 and 1993. Names and other personal identifiers common to the DMDC and Vital Statistics Registries will be matched with Birth Defects Registry of each state. Overall and diagnostic-specific rates of congenital anomalies will be calculated and compared among infants of GWV and NDV and the general population (excluding GWV and NDV) of each state. Univariate analysis will be applied to compare rates between subpopulations. Multivariate techniques will be applied to identify characteristics associated with congenital malformations. Cluster analysis will be performed to detect interrelations among selected anomalies and subpopulations.

EXPECTED PRODUCTS (MILESTONES): The overall as well as diagnostic-specific prevalence rates of congenital anomalies among infants of GWV and NDV will be determined. Pathologic patterns of congenital malformations among infants of GWV will be described, and may serve useful in developing hypotheses about genetically or environmentally-induced birth defects for future investigations.

STATUS/RESULTS TO DATE: Data provided by the Hawaii Birth Defects Monitoring Program has been analyzed to compare overall and diagnostic-specific rates of birth defects. Birth defect data among infants of GWV and NDV in Arizona are complete for 1989-1991; the Arizona Birth Defect Monitoring Program is still collecting data for 1992-1993 births. Identification of births to military personnel in Arkansas and Metropolitan Atlanta is complete. Linkage to the Arkansas Birth Defect Monitoring Program data is in progress to identify military infants with birth defects. Birth defect data provided by the Metropolitan Atlanta Birth Defects Program is being analyzed.

PUBLICATIONS: Destiche DA. Aranata MRG. Schlangen KM. Horiuchi BY. Onaka AT. Gray

GC. Deterministic Linkage to Identify Births to Military Personnel Using Hawaii Birth Certificate and Military Personnel Records. 1998 Conference of Federally Sponsored Gulf War Veterans' Illnesses Research, Arlington, Virginia.

Schlangen KM, Araneta MRG, Destiche DA, Zau AC, Kaiser KS, Gray GC. Evaluating Birth Defects among Infants Born to Persian Gulf War Veterans. 1998 Conference of Federally Sponsored Gulf War Veterans' Illnesses Research, Arlington, Virginia.

Kaiser KS, Araneta MRG, Destiche DA, Schlangen KM, Zau AC, Gray GC. A Comparison of Birth Defect Rates in Arizona Before the Persian Gulf War, 1989-1991. 1998 Conference of Federally Sponsored Gulf War Veterans' Illnesses Research, Arlington, Virginia

Title: Physiological and Neurobehavioral Effects in Rodents from Exposure to Pyridostigmine, Fuels, and DEET

Project #: DoD-2

Agency: DoD

Study Location: Tri-Svc Tox Progr, NMRI-TD

Project Status: Complete

Research Type: Mechanistic

P.I.: J Rossi, Ph.D.

Research Focus: Interactions, Pyridostigmine Bromide, Brain & Nervous System

Start Date (CY): 1994

Est. Completion (CY): 1997

OVERALL PROJECT OBJECTIVE: This project has evaluated the potential of a simulated Gulf War (GW) exposure consisting of multiple chemicals, alone and in conjunction with an imposed psychological stressor, to induce biological effects in Sprague-Dawley rats. Effects were for similarity to symptoms and effects reported by exposed Gulf War veterans (e.g., "Gulf War Veterans' Illnesses" (GWVI)). No animal model for GWI currently exists; therefore, the project investigated if the rodent model can reproduce the symptoms reported in GWI.

SPECIFIC AIMS: This study tested the hypothesis that simultaneous exposure to a combination of chemical stressors, each well tolerated individually, causes subtle physiological changes when coupled with psychological stress. The study used Sprague-Dawley rats as an animal model to identify effects reported by humans similarly exposed during the GW. The study also attempted to identify biomarkers for use in evaluating the extent of exposure and of effect in humans.

METHODOLOGY: The chemical stressors and routes of exposure evaluated were: inhalation of fuel vapor (diesel, jet fuel) and their combustion products, dermal absorption of the insect repellent, N, N-diethyl-m-toluamide (DEET), and oral (or i.p.) administration of pyridostigmine bromide (PB). Psychological stress was simulated by intermittent administration of a mild electrical shock. The shock was administered randomly by a computer controlled apparatus, so that it would be uncontrollable and unpredictable, functioning as a traumatic stressor. Male Sprague-Dawley rats were exposed by inhalation to diesel and/or jet fuel vapor, orally to PB, and dermally to DEET, alone, and in various combinations. Half of the animals received a periodic, random, mild electrical shock as psychological and neurobehavioral changes by standard tests, immediately after exposure and after a 60-day latent period. The study investigated physiological and neurobehavioral effects, neurotransmitter levels, and changes in protein composition in serum/plasma, liver, kidney, testes, and brain from exposed rats.

EXPECTED PRODUCTS (MILESTONES): This study evaluated the potential of a simulated GW exposure consisting of multiple chemical exposures, alone and exacerbated by an imposed psychological stressor, to induce toxic effects in Sprague-Dawley rats which may have some similarities to the symptoms reported by Gulf War veterans. This study investigated, in a rodent model, factors that may have contributed to the development of symptoms reported in GWI. Representative chemical exposures from the GW theater, with and without an imposed psychological stressor, were investigated individually and in combinations to determine possible interactive effects.

STATUS/RESULTS TO DATE: Almost all of the significant differences in this study involved

animals exposed to JP4 fuel vapor only, or in combinations of JP4 or footshock and pyridostigmine bromide and DEET. Differences between groups in the 14 day neurobehavioral study were found only for startle (JP4, stress, stress & JP4) and for appetite reinforcer approach (JP4). In the 60 day study, differences were observed in a variety of measures on the Navy Neurobehavioral Toxicity Assessment Battery (NTAB) for combinations of stressors which included JP4 or footshock. The results suggest the JP4 fuel vapor and/or JP4 in combination with footshock stress affect a variety of neurobehavioral tests. A manuscript is being prepared for peer-reviewed publication of these results.

PUBLICATIONS: Nelson RA. Determining Types of Health Effects to Persian Gulf Veterans Due to Exposure to Occupational Hazards, Thesis No. AFIT/GEE/ENV/95D-13, Air University, USAF Institute of Technology, Wright-Patterson Air Force Base, OH, 1995.
Rossi J, MacMahon K, Ritchie G, Wolfe R, Leahy H, Nordholm A, Caldwell D.
Neurobehavioral changes in rats exposed to a simulated Persian GW environment Society of Toxicology meeting, Cincinnati, Ohio, March 1997 (abstract).
MacMahon K, Rossi J, Wolfe R, Leahy H, Narayanan L, Witzmann F, Eggers J, Ritchie G, Nordholm A. Physiological responses of Sprague-Dawley rats exposed to low doses of pyridostigmine bromide, N,N-diethyl-m-toluamide (DEET), JP-4 jet fuel, and stress. Society of Toxicology meeting, Cincinnati, Ohio, March 1997, Abstract.

Title: The General Well-Being of Gulf War Era Service Personnel from the States of Pennsylvania and Hawaii: A Survey

Project #: DoD-4

Agency: DoD

Study Location: WRAIR, Wash. DC

Project Status: Complete

Research Type: Epidemiology Research

P.I.: David Marlowe, Ph.D.

Research Focus: Symptoms/General Health

Start Date (CY): 1992

Est. Completion (CY): 1994

OVERALL PROJECT OBJECTIVE: Assess the general sense of well-being of Gulf War era veterans in the States of Hawaii and Pennsylvania.

SPECIFIC AIMS: Identify groups within the population reporting physical or mental distress and highlight probable causes or contributing factors. Ascertain whether any group within this population was at risk for future development of PTSD related to their Gulf War era experiences. Evaluate the status of the 14th Quartermaster Detachment. Assess the impact of the casualties of the 14th Quartermaster Detachment on the community of Greensburg, Pennsylvania.

METHODOLOGY: Citizens of Pennsylvania and Hawaii who served during the period of Operation Desert Shield/Storm were identified and asked to participate in a survey involving questionnaires and interviews (approx. 14,100). Study materials were designed and administered; 4,334 responses were obtained. Data was integrated with responses from an additional 25,000 individuals being studied as part of a larger assessment. Survey instruments and methods included a questionnaire, Impact of Events Scale, Brief Symptom Inventory, PTSD Risk Algorithm, and records/reports from Pacific Center for PTSD and from PTSD Clinical Team, VA Medical Center, Pittsburgh, PA. The distribution of psychological and illness symptoms in the samples were analyzed, and differences in psychological and physical symptoms between deployers and nondeployers were examined.

EXPECTED PRODUCTS (MILESTONES): An assessment of the general physical and mental well-being of the subject population. Information to assist Congressional determination of the need for appropriated funds for specialized counseling and support services to counter any adverse effects of service during Operation Desert Shield/Storm. Information addressing potential

links between service in the Gulf and subsequent health and adjustment difficulties upon return.

STATUS/RESULTS TO DATE: The studied population was generally well-adjusted, with subgroups experiencing either physical symptoms, high levels of stress, or both (about 15% of the population). Among those deployed and who reported physical symptoms, neither exposure to combat nor its aftermath bear much relationship to their distress. Only the fact of deployment explains the higher rate of physical symptoms compared those who were not deployed. About 15% of those deployed were experiencing both stress and physical symptoms at the time of the study. Many of these seem at risk for development of further difficulty, particularly PTSD.

PUBLICATIONS: Stretch RH, Bliese PD, Marlowe DH, et al. Physical health symptomatology of Gulf War-era service personnel from the states of Pennsylvania and Hawaii. *Military Medicine*, 160:131-136, 1995.

Stretch RH, Bliese PD, Marlowe DH, et al. Psychological health of Gulf War-era military personnel. *Military Medicine*, 161:257-261, 1996.

Stretch RH, Marlowe DH, Wright KM, et al. Post-traumatic stress disorder symptoms among Gulf War veterans. *Military Medicine*, 161:407-410, 1996

Title: Program DoD-6

Project #: DoD-6

Agency: DoD

Study Location: WRAIR, Wash. DC

Project Status: Ongoing

P.I.: James Meyerhoff, M.D.

This is the parent Program for DoD projects DoD-6A through DoD-6B.

PUBLICATIONS: none to

Title: Combat Stress Pharmacotherapy

Project #: DoD-6A

Agency: DoD

Study Location: WRAIR, Wash. DC

Project Status: Ongoing **Research Type:** Mechanistic

P.I.: James Meyerhoff, M.D.

Research Focus: Brain & Nervous System, Treatment

Start Date (CY): 1988

Est. Completion (CY): 1999

OVERALL PROJECT OBJECTIVE: Combat stress reaction (CSR) and posttraumatic stress disorder (PTSD) are thought to be part of a common spectrum of disorders. It is essential to develop animal models for combat stress reaction, to identify neurochemical mechanisms of, and pharmaceutical countermeasures for combat stress effects. Emphasis is on rapidly acting countermeasures which could be administered far forward, and which would allow the stress casualty to remain on duty and prevent the development of PTSD. Pharmaceutical countermeasures must be free of unwanted side effects, such as sedation or impairment of memory or performance.

SPECIFIC AIMS: Evaluate clinical and pre-clinical pharmaceuticals to prevent/reverse conditioned defeat (CD). Use acoustic startle, the swim test, neuroendocrine markers and telemetric monitoring of heart rate in behaving, unrestrained animals exposed to threat, develop technology for predicting vulnerability to conditioned defeat; thus enhancing evaluation of possible preventive measures.

EXPECTED PRODUCTS (MILESTONES): FY 97: Examine the effect of valium on CD in another species (mice) to test the generality of the finding that valium may exacerbate CD (and might be

contraindicate in combat stress reaction). Extend testing to include benzodiazepine antagonists and inverse agonists in mice. In addition, begin testing Buspirone, a clinically available, non-sedating, non-benzodiazepine anxiolytic drug in mouse CD model. Will also test its more potent pre-clinical analogs - Gepirone, ipsapirone, and tiaspirone. The startle response has been reported to be exaggerated in both CSR and PTSD. Accordingly, we will test the effect of CD on acoustic startle in the mouse. To identify potential aids to diagnosis of risk for CSR, we will further characterize hormonal responses to CD. FY 98: Begin testing other behaviorally active clinically-available drugs in mouse CD model: (a) beta blockers (atenolol); (b) antidepressants used in panic disorders (Prozac, tricyclics); (c) stimulants such as caffeine and amphetamine. Evaluate all listed compounds for capability to reverse CD-induced deficits in the swim test. FY 99: Begin testing novel pre-clinical, non-benzodiazepine anxiolytics in the mouse CD model, including cholecystokinin antagonists; corticotrophin releasing hormone antagonists; and Neuropeptide Y (NPY) agonists. Evaluate potential therapeutic effects of thyrotropin releasing hormone (TRH) - an endogenous peptide stimulant successfully employed to reverse learned helplessness. Target drug intervention at specific brain sites, study viable brain slices from defeated mice, including amygdala and hippocampus, to evaluate agents for selective effects on specific circuits involved in CD. Study the effects of CD on cognitive function and neurodegeneration.

STATUS/RESULTS TO DATE: By establishing a streamline model of conditioned defeat (CD), we have met the requirement of developing an animal model for testing diagnostics for component processes of combat stress reaction (CSR) and screening drugs for safe, rapid treatment. We have shown that CD markedly decreases activity in the swim test - a measure used in the pharmaceutical industry to screen antidepressants. In an effort to develop reliable predictors and clinical indicators for CSR, we have characterized the dramatic responses to CD of hormones stimulated via the anxiogenic peptide, corticotrophin releasing hormone (CRH). We have shown that acoustic startle response (ASR) in hamsters was exacerbated by administration of CRH. This is significant because exaggerated acoustic startle response has been validated as a biological marker for PTSD and reported as a symptom of CSR as well. We found that valium is clearly counterproductive as a treatment for hamsters, as it markedly exacerbated CD. Thus, the CD model affords us the capability of identifying drugs which might be contraindicated, as well as those which might be beneficial. By way of confirmation, an Israeli colleague is reporting that benzodiazepines fail to block the development of PTSD following trauma. We have optimized the CD model as a test bed for screening drugs by modifying it to deliver rapid, high-volume, low-cost throughout.

PUBLICATIONS: Hebert M, Potegal M, Moore T, Evenson AR, Meyerhoff J. Diazepam exacerbates conditioned defeat in hamsters (*Mesocricetus auratus*). *Pharmacology, Biochemistry and Behavior*, 55:405-413, 1996.
Potegal M, Ferris C, Hebert M, Skaredoff L, Meyerhoff J. Attack priming in female Syrian golden hamsters is mediated by a c-fos coupled process within the corticomedial amygdala. *Neuroscience*, 75:869:880, 1996

Title: Combat Stress Diagnosis, PTSD Prevention

Project #: DoD-6B

Agency: DoD

Study Location: WRAIR, Wash. DC

Project Status: Complete **Research Type:** Clinical Research

P.I.: James Meyerhoff, M.D.

Research Focus: Brain & Nervous System, Diagnosis Prevention

Start Date (CY): 1987

Est. Completion (CY): 1998

OVERALL PROJECT OBJECTIVE: Develop diagnostic instruments for far-forward, early diagnosis of soldiers at risk for combat stress reaction (CSR) and posttraumatic stress disorder

(PTSD) to allow early intervention to prevent chronic PTSD.

SPECIFIC AIMS: Use combined neuroendocrine and psychophysiological techniques to develop rapid, reliable and inexpensive means of determining soldiers' level of stress, fitness for duty and risk of CSR/PTSD.

METHODOLOGY: We will measure salivary levels of cortisol, testosterone, and dehydroxyepiandrosterone sulfate (DHEAS) as stress indices. We will critically evaluate the reliability of voice frequency modulation as a stress index. In collaboration with the New Haven VA Medical Center we will conduct a prospective study on risk of stress reactions/PTSD in Special Operations Forces.

EXPECTED PRODUCTS (MILESTONES): FY 96: Correlate salivary cortisol and Soldier of the Month Board performance; measure effect of stress on salivary testosterone and DHEAS. FY 97: Determine effect of psychological stress on voice frequency modulation spectrum and correlate with performance as well as physiological and hormonal changes. FY 98: Complete prospective study of changes in acoustic startle and dexamethasone suppression tests (using salivary cortisol measurements) in soldiers subjected to stressful deployments.

STATUS/RESULTS TO DATE: Ongoing. Because the "Soldier of the Month (SOM) Board" is a uniquely robust physiological stressor, we have been using it as a test bed to develop and validate field-ready stress measures, such as a battery of non-invasive (salivary) hormonal measures. We will use these measures in stress studies in soldiers at risk for combat stress reaction and/or PTSD during military operations in the field. We have characterized the psychoendocrine and autonomic profile of operational stress, using the SOM Board as a model. Elevated plasma and salivary cortisol levels were measured in soldiers after an SOM Board experience. We have identified several plasma hormones which correlate positively with anxiety, and have demonstrated that anticipatory anxiety predicts poor performance.

PUBLICATIONS: Pitman RK, Orr SP, van der Kolk BA, et al. Analgesia: a new dependent variable for the biological study of PTSD, In Post Traumatic Stress Disorder: Etiology Phenomenology and Treatment (ed by Wolf ME). American Psychiatric Press, 141-147, 1990.

Title: Health Risk Assessment of Embedded Depleted Uranium: Behavior, Physiology, Histology, and Biokinetic Modeling

Project #: DoD-7A

Agency: DoD

Study Location: AFRRRI, Bethesda, MD

Project Status: Complete

Research Type: Mechanistic

P.I.: Terry Pellmar, Ph.D.

Research Focus: Depleted Uranium, Environmental Toxicology

Start Date (CY): 1994

Est. Completion (CY): 1998

OVERALL PROJECT OBJECTIVE: Evaluate health risks associated with tissue-embedded depleted uranium (DU) fragments by studying the behavioral, physiological, and histological consequences of implanted DU in a rodent model.

SPECIFIC AIMS: Evaluations in the DU-implanted rodent model included: behavioral tests of motor activity and memory, conduction studies of peripheral nerve function, measurement of central nervous system excitability in brain slice preparations, biochemical assessments of renal function, histological assessment of local tissue damage and capsule formation, measurement of tissue concentrations of uranium, and development of a biokinetic model describing the distribution of uranium over time.

METHODOLOGY: Appropriate doses to be used for analysis of DU effects were defined and then a study of 325 rats was conducted to obtain toxicity data. Rats were randomly assigned to 5 treatment groups: 1) low-dose DU. 2) medium-dose DU. 3) high-dose DU. 4) tantalum (Ta)

controls, and 5) non-implanted sham-surgical controls. In the low-dose and medium-dose groups, Ta was substituted for a fraction of the DU pellets to keep the total number of implanted fragments constant. Rats were evaluated, euthanized and tissue samples taken at 1, 6, 12, or 18-months following fragment implantation. The investigators determined that sample sizes of 15 rats per group were necessary to achieve statistical significance, and additional animals (20 rather than 15) were implanted for the 18-month time point with the expectation of a 20-25% natural mortality. Each rat was thoroughly evaluated for changes in behavior (functional battery, motor activity, passive avoidance test), peripheral nerve function (conduction velocity measurements), CNS excitability (hippocampal brain slice electrophysiology), renal function (plasma and urine biochemistries) and tissue histology including capsule formation. In addition, data on tissue uranium levels (measured by kinetic phosphorescence analysis) from a subgroup of rats was used to develop a biokinetic model to predict uranium distribution. All measured parameters were compared among groups through analysis of variance and significance accepted at the $P < 0.05$ level.

EXPECTED PRODUCTS (MILESTONES): Determine appropriate doses for a DU toxicity study in the rat: July 1995. Complete toxicity assessment at the one-month point: Dec 1995. Complete toxicity assessment at the six-month point: Dec 1996. Complete toxicity assessment at the 12-month point: June 1997. Complete toxicity assessment at the 18-month point: Dec 1997. Complete biokinetic modeling for distribution of DU fragments: Jan 1998.

STATUS/RESULTS TO DATE: Throughout the 18 months of exposure to DU fragments, uranium levels were high and dose-dependent in kidney, urine, and bone. Despite high uranium levels in kidney, no renal toxicity was evident. Other tissues including muscle, spleen, liver, heart, lung, lymph nodes, and testicles contained significant concentrations of uranium in implanted animals. Unexpectedly, uranium was found in the brain of DU-implanted animals. Uranium was not uniformly distributed throughout the brain. Excitability of the hippocampus was modified in the DU-implanted animals at 6 and at 12 months. By 18 months after fragment implantation, it appeared that the effects of aging and of DU exposure converged, thereby obscuring the effect of the metal. Although simple behavior tasks did not reveal any gross performance decrements associated with DU exposure, future studies using more complex learning tasks may reveal subtle cognitive deficits. In comparison to Ta controls, DU-implanted animals did not show any histopathology in microscopically examined tissues. At 18 months, however, DU appeared to cause a mild stimulation of hematopoiesis as evidenced by hyperplasia of bone marrow and both the red and white pulp of the spleen. Examination of the pellets in situ revealed fibrous tissue adhering to the DU but not the Ta pellets. Body weight in high-DU dose animals was significantly lower than controls. A biokinetic analysis of the uranium redistribution in the implanted rats suggested that the existing model of the International Commission for Radiological Protection (ICRP) is adequate for assessment of risk from uranium fragments.

PUBLICATIONS: Hogan J, Benson KA, Landauer M, Pellmar TC. Toxicity of embedded Depleted Uranium (DU) in the rat. *The Toxicologist*, 36:17, 1997.

Pellmar TC, Hogan J, Benson KA, Landauer M. Health Risk Assessment of *Persian Gulf Veterans' Illnesses Research Database - Project No DoD-7A* Embedded Depleted Uranium (DU) 6-Month Evaluation Point, AFRRRI Special Publication Report, 97-4, 1997.

Miller AC, Emond C, Whittaker T, Xu J, Strocko S, Hogan J, Page N, Pellmar T. Urinary and serum mutagenicity studies with rats implanted with depleted uranium or tantalum pellets. 29th Annual Mutagenesis Society Meeting, Anaheim, CA, 1998.

Benson KA, Pellmar TC. Neurotoxicity and reproductive effects of embedded depleted uranium in the rat. Conference of Federally Sponsored Gulf War Veterans' Illnesses Research, Pentagon City, VA, p. 109, June 1998.

Pellmar TC, Hogan JB, Benson KA, Landauer MR. Depleted uranium distribution and toxicology in a rodent model. AFRRRI Special Publication Report, 98-3:7-9, 1998.

Miller AC, Fuciarelli AF, Ejnik EJ, Strocko S, Hogan J, Page N, Pellmar T. Urinary and serum mutagenicity studies with rats implanted with depleted uranium or tantalum pellets. *Mutagenesis*. 13:643-648. 1998.

Pellmar TC, Fuciarelli AF, Ejnik JW, Hamilton M, Hogan J, Strocko S, Emond C, Mottaz HM, Landauer MR. Distribution of uranium in rats implanted with depleted uranium pellets. Toxicological Sciences (in press)

Title: Carcinogenicity of Depleted Uranium Fragments

Project #: DoD-7B

Agency: DoD

Study Location: Inhal Tox Lab, Albuquerque NM

Project Status: Ongoing

Research Type: Mechanistic

P.I.: Fletcher Hahn, Ph.D.

Research Focus: Depleted Uranium, Environmental Toxicology

Start Date (CY): 1995

Est. Completion (CY): 1999

OVERALL PROJECT OBJECTIVE: Assess the carcinogenic risks associated with long-term exposure to DU-containing shrapnel in wounds using a rodent model.

SPECIFIC AIMS: Determine the carcinogenicity of radioactive DU fragments in tissues relative to nonradioactive (tantalum) foreign-body fragments, and correlate urine and kidney concentrations of uranium with time after implantation of DU fragments.

METHODOLOGY: Relative carcinogenicity of embedded DU fragments is being determined using test metals, in the form of flat squares or pellets, surgically implanted into the muscles of rats. Thorotrast was used as a positive control radioactive material. These animals will be observed for two years for the onset, incidence, and biological characteristics of tumors developing at the site of implantation. The incidence of subcutaneous tumors will be compared among dose groups by using a Cox proportional hazards model.

Renal toxicity of chronic uranium exposure will be studied after determination of the time course to achieve a steady-state renal DU concentration from an implanted source. Urinalysis will include U concentration and biochemical indicators of kidney function. Rats will be examined for lesions, with particular attention to the urinary system. Kidneys will be examined histologically for lesions related to U toxicity. U content in the kidney and skeleton and remainder of the carcass will be determined. Laser phosphorimetry is being used to analyze tissue and urine samples for U.

EXPECTED PRODUCTS (MILESTONES): The carcinogenic hazard of radioactive DU fragments relative to nonradioactive fragments is being determined so that informed judgments can be made about the clinical management of veterans with DU fragments embedded in their soft tissues. Milestones of this study are: May 1997: Initiation of the long-term study of potential carcinogenic effects of implanted DU fragments. May 1999: Completion of the long-term carcinogenesis study of DU fragments.

STATUS/RESULTS TO DATE: The in vitro dissolution of DU and DUTi fragments in serum ultrafiltrate (SUF) and acid solution (pH5) has been determined using a static dissolution cell. The DUTi dissolved more slowly than the DU in SUF, with a half time of 277 days compared with 163 days for DU. Both metals had higher dissolution rates in pH5. This study will aid in understanding the dissolution mechanisms for DU. Using 3 sizes resulted in rats receiving a mass of metal that ranged across a factor of 20 and a surface radioactivity that ranged a factor of 10. In addition, a group of rats received tantalum fragments of similar sizes to serve as negative control animals, and a group of rats received Thorotrast injections to serve as positive control animals for radioactive materials.

A carcinogenesis bioassay protocol was initiated to study the carcinogenesis potential of DUTi. Fragments of DU (0.75% Ti) in 3 sizes were implanted into the leg muscles of rats. To date, 15 months after implantation, no neoplasms related to the implants have developed. The rats will be observed for nine more months before terminal sacrifice.

PUBLICATIONS: Hahn FF. Lundgren DL. Hoover MD. Guilmette RA. DU Distribution and

Carcinogenesis Studies. In Health Effects of Embedded Depleted Uranium Fragments, Livergood OR, ed. An Armed Forces Radiobiology Institute Workshop, 1996, AFFRI Special Report Publication 98-3, National Technical Information Service, 1998.

Title: Program DoD-8
Project #: DoD-8
Agency: DoD
Study Location: WRAIR, Wash. DC
Project Status: Ongoing
P.I.: COL Rodney Michael
This is the parent Program for DoD projects DoD-8A through DoD-8B.
PUBLICATIONS: none to

Title: Serologic Diagnosis of Viscerotropic Leishmaniasis (VTL)
Project #: DoD-8A
Agency: DoD
Study Location: WRAIR, Wash. DC
Project Status: Complete
Research Type: Development
P.I.: COL Rodney Michael, M.D.
Research Focus: Leishmaniasis, Diagnosis
Start Date (CY): 1993
Est. Completion (CY): 1996
OVERALL PROJECT OBJECTIVE: Develop a reliable serologic test for viscerotropic leishmaniasis.
SPECIFIC AIMS: The goal was to identify an antibody (Ab)-based serologic assay to detect active infection with Leishmania parasites causing the clinical syndrome of VTL using a standard format such as enzyme-linked immunosorbent assay (ELISA).
METHODOLOGY: Use of ELISA-based assays to diagnose active infection with an infectious agent is a widely accepted and useful diagnostic intervention. Currently, there are no commercially available serologic assays to detect Leishmania infection. Some specialty labs use the indirect immunofluorescence technique (IFAT) format to detect antibodies to surface proteins of the promastigote (insect vector form of the parasite); however, the IFAT is not useful in VTL. Once the parasite is injected into humans by the sand-fly vector, it transforms into an amastigote (the form found in mammals). The amastigote is a genetically different morphologic form which expresses a unique repertoire of antigens. This effort is based on the hypothesis that these amastigote dominant antigens hold the key to serologic diagnosis of VTL. Using recombinant molecular biology techniques, proteins which are recognized by VTL sera can be made in large quantities and used as capture antigens in ELISA tests.
EXPECTED PRODUCTS (MILESTONES): Identification of reactive clones made from screening a genomic library of Leishmania DNA, cloning and sequencing the recombinant proteins and using the recombinant proteins to screen sera from patients with viscerotropic leishmaniasis (VTL).
STATUS/RESULTS TO DATE: A genomic library was made from a Desert Storm isolate of Leishmania. Parasite DNA was sheared and introduced into E. coli via phage virus. Bacterial clones with parasite DNA were transferred to nitrocellulose and then incubated with radioiodinated protein labeled VTL sera. Reactive clones were then isolated, induced, and protein was sequenced. Patients with VTL appear to recognize a 210 kd recombinant protein, called Lt-1, the carboxy third of the protein is made of repeats. Smaller recombinant proteins and synthetic peptides based on the repeats have been made. To date, none of these peptides reliably

discriminates infected from noninfected samples.

PUBLICATIONS: Dillon DC, Day CH, Whittle JA, Magill AJ, Reed SG. The molecular and immunological characterization of Leishmania tropica antigens: serological and cellular responses by viscerotropic Leishmaniasis Patients. VIII Intern Congress of Parasitology, 1994. Dillon DC, Day CH, Whittle JA, Magill AJ, Reed. The Molecular and immunological characterization of Leishmania tropica antigens: serological and cellular responses by Viscerotropic Leishmaniasis Patients. 43rd Annual Mtg AM SocTrop Med & Hygiene, 1994. Dillon DC, Day CH, Whittle JA, Magill AJ, Reed. Characterization of a Leishmania tropica antigen that detects immune responses in Desert Storm Viscerotropic Leishmaniasis patients. Proc Natl Acad Sci, (92) :7981-82, 1995

Title: Development of a Leishmania Skin Test Antigen (LSTA)

Project #: DoD-8B

Agency: DoD

Study Location: WRAIR, Wash. DC

Project Status: Ongoing

Research Type: Development

P.I.: MAJ Robert Miller

Research Focus: Leishmaniasis, Diagnosis

Start Date (CY): 1993

Est. Completion (CY): 2005

OVERALL PROJECT OBJECTIVE: Develop a reliable skin test for Leishmania infection.

SPECIFIC AIMS: The goal is to identify a safe, potent, and non-sensitizing LSTA; manufacture it under cGMP; and obtain an IND for its use in phase I and phase II clinical trials, and ultimately a commercially available, FDA-licensed product.

METHODOLOGY: Skin tests are widely accepted diagnostic interventions for diagnosis of prior infection with an infectious agent (e.g., tuberculosis). Currently there is no Leishmania skin test licensed for use in the USA or available under an IND. Once required phase I and phase II studies are completed in humans, studies could be performed in Gulf War veterans with confirmed and suspected leishmaniasis.

EXPECTED PRODUCTS (MILESTONES): Phase I/II trials of LSTA in FY00; Phase III trial in FY02; Licensure by FY05.

STATUS/RESULTS TO DATE: Microfluidized-lysate (MFL)-LSTA was previously manufactured under cGMP in the WRAIR pilot production facility and tested in phase I trial after approval of an IND by the FDA. Sixty-six doses of MLF-LSTA were administered to 15 volunteers. The product was well tolerated, however, two volunteers exhibited a type I allergic cutaneous reaction to the antigen and placebo formulation. The most likely cause of this reaction was pre-existing hypersensitivity to dextran, a component of the lyophilization buffer. To avoid such problems, a reformulation of LSTA lacking dextran was undertaken, under cGMP, at the WRAIR pilot production facility in FY98. This new formulation will be retested; an abbreviated phase I (one dose) and a phase IIa dose ranging and potency trials are currently planned.

PUBLICATIONS: Stiteler JM, Rowton Ed, Grogl M, Eckels KH, Martin SK, Miller R. Current Good Manufacturing Practices (cGMP) Production of a Heat-Treated Leishmania Skin Test Antigen, MFL-LSTA(R2). Abstract, 47th Annual Meeting of the American Society of Tropical Medicine and Hygiene, OCT 18-22, 1998, San Juan, Puerto Rico.

Title: Identification of the Genetic Factors Which Control Tropism in Leishmania

Project #: DoD-9

Agency: DoD

Study Location: WRAIR, Brazilia

Project Status: Complete

Research Type: Mechanistic

P.I.: LTC Max Grogil, Ph.D.

Research Focus: Leishmaniasis, Treatment

Start Date (CY): 1994

Est. Completion (CY): 1998

OVERALL PROJECT OBJECTIVE: Identify the gene(s) that control tropism in Leishmania and Determine its (their) sequence and function.

SPECIFIC AIMS: The goal of this research is to identify the gene(s) that control tropism in Leishmania. The identification of a "tropism" gene will enable the development of specific gene probes (primers) to be used in a patient screening program to identify those at risk of reactivation of latent infections; address the fundamental question of infectious disease pathophysiology, namely why an organism infects a particular cell; optimize treatment regimens according to Leishmania species and the immune status of the host. The identification of the genetic factor(s) involved in the visceralization of Leishmania will require development in vitro and in vivo models of tropism to facilitate the study of viscerotropic leishmaniasis (diagnosis, therapy, prevention) and Leishmania tropism.

METHODOLOGY: Develop in vitro and in vivo models of Leishmania tropism to use for the determination of the genetic factors controlling tropism: a promastigote temperature sensitivity model; and a cutaneous and a visceral animal model of Leishmania tropism. Create Leishmania mutants with altered tropism in the in vitro and in vivo models of tropism: temperature sensitive parasites changed to resistant and visa versa; and cutaneous parasites (in the animal model) changed to visceral and visa versa. Use recently developed molecular genetic techniques to restore the original (wild-type) phenotype to the Leishmania temperature and tropism mutants by transfection with the appropriate Leishmania cosmid DNA libraries. Identify the gene(s) controlling temperature sensitivity and tropism present in the cosmid(s) which restore(s) a wild-type phenotype to the Leishmania cells. Retest the function of these genes in both models using transfection

Methodology.

EXPECTED PRODUCTS (MILESTONES): Molecular characterization includes Identification of the genes present in the cosmids which restore a wild-type phenotype to the Leishmania mutants, sequencing the genes by 1998, and determining the homologies with genes in the database by 1998.

STATUS/RESULTS TO DATE: An in vitro promastigote temperature model was developed and the investigators determined that temperature sensitivity in vitro correlates with Leishmania tropism in vivo. There is no absolute temperature requirement for dermatropism, but there is a minimum temperature resistance requirement for visceralization. The parasite strains that have an unusual tropism in the human host (to include the viscerotropic *L. tropica* from Desert Storm) also show an unexpected temperature sensitivity in the model. Two animal models were developed where the tropism of the Leishmania strain is known, uniform, and reproducible (all organisms are found in one location, either visceral or cutaneous) when inoculated in the skin (sc) to mimic a sand fly bite, including a cutaneous model with no visceralization, and a visceral model with no skin lesions. Leishmania temperature sensitive and resistant mutants have been obtained. Laboratory techniques were developed and standardized for transfection and for the Leishmania cosmid DNA library. An *L. donovani* and two *L. tropica* temperature-resistant strains were selected in vitro. An *L. mexicana* strain was chemically mutagenized and a tropism mutant was selected in our hamster model. Wild-type DNA cosmid libraries were made of all the Leishmania strains (4) to be mutagenized and selected for altered tropism in the hamster model. Both the in vitro and in vivo models developed to do the molecular biology studies have been published. The most relevant issue of viscerotropic leishmaniasis for Desert Storm was that *L.*

tropica, which should be solely dermatropic, was found in viscera of veterans. The study identified an important question for further research prior to termination due to lack of funding: the identification of genetic factors which control tropism in leishmaniasis.

PUBLICATIONS: Callahan HL, Portal IF, Grogl M. Development of an in vivo Leishmania temperature

Callahan HL, Portal IF, Bensinger SJ, Grogl M. The temperature sensitivity of Leishmania promastigotes in vitro: a model for tropism in vivo. Exp. Parasit, 84:400-409, 1996.

Callahan HL, Grogl M. Development of animal models to study Leishmania tropism at the molecular level. Am J Tropical Med. (in press) sensitivity model: preliminary studies with strains with aberrant tropism in vivo. 44th Annual Meeting of Am Soc Tropical Med & Hygiene, 53(2):215, 1995

Title: Pyridostigmine Synergistic Toxicity Study

Project #: DoD-10

Agency: DoD

Study Location: CHPPM, Aberdeen MD

Project Status: Complete

Research Type: Mechanistic

P.I.: Wilfred McCain, Ph.D.

Research Focus: Pyridostigmine Bromide, Interactions

Start Date (CY): 1994

Est. Completion (CY): 1994

OVERALL PROJECT OBJECTIVE: Determine potentially toxic interactions when pyridostigmine bromide, permethrin, and DEET are given concurrently to male rats by gavage.

SPECIFIC AIMS: Phase I determined the acute oral lethal dose response relationship of each compound with the propylene glycol solution in which it was delivered. Phase II was a dose response study using results from Phase I as well as calculated dosage solutions of the three materials in combinations. This study was motivated by concerns about the possible synergism of pyridostigmine taken by the service members in ODS to protect them against potential nerve agent exposure and permethrin and DEET, insecticides which were used at the same time.

METHODOLOGY: The first phase was the development of acute oral lethal dose-response relationship of each compound with the vehicle, determine dosage levels for the second phase. Part 1 of Phase II served as a positive control for the interaction portion of the study and verified data obtained from Phase I. The Part 1 dose response study used dosing developed from Phase I. Part 2 of Phase II was similar to the control portion except that the vehicle contained the calculated LD16 of the other two compounds. The Phase I study protocol used 16 groups of 10 animals each, with one group of 10 receiving only the propylene glycol vehicle. Gross necropsy was performed on all animals as soon as possible after death. Animals surviving the 14 day study period were sacrificed and examined for gross pathological lesions. The Phase II study used 15 groups of six animals each for the positive control portion and 18 groups of 10 animals each for the interaction portion. Animals were necropsied and examined for gross pathological lesions.

EXPECTED PRODUCTS (MILESTONES): Report on toxicity produced by high dose interactions of military materiel and medical pretreatments.

STATUS/RESULTS TO DATE: The principal finding was that at extraordinarily high doses, there is an increased mortality in rats given pyridostigmine bromide, permethrin, and DEET simultaneously by gavage when compared to expected additive lethal effect of the individual compounds. The direct significance of these findings to ground forces who served in the Gulf War is uncertain because these doses and route of exposure do not correspond to any suspected human exposures.

PUBLICATIONS: US Army Center for Health Promotion and Preventive Medicine Report. Acute Oral Toxicity Study of Pyridostigmine Bromide, Permethrin, and DEET in the Laboratory Rat. Toxicological Study, 1995, 75-48-2665

Title: Male/Female Differential Tolerances to Pyridostigmine Bromide

Project #: DoD-11

Agency: DoD

Study Location: S.FL Drug Res Miami FL

Project Status: Complete

Research Type: Clinical Research

P.I.: Kenneth Lasseter, M.D.

Research Focus: Pyridostigmine Bromide

Start Date (CY): 1994

Est. Completion (CY): 1998

OVERALL PROJECT OBJECTIVE: Determine if males and females have different tolerances to doctrinal dose (30 mg every 8 hours) of pyridostigmine bromide.

SPECIFIC AIMS: To evaluate the tolerance of pyridostigmine bromide (30 mg every 8 hours for 21 days); to evaluate multiple dose kinetics; and to evaluate the effect of weight in males and females upon drug tolerance.

METHODOLOGY: Double-blind study.

EXPECTED PRODUCTS (MILESTONES): Final Report.

STATUS/RESULTS TO DATE: In-life portion of the study and the one year follow-up have been completed. Results from this study indicate that pyridostigmine is safe. Expected side effects, primarily gastrointestinal, were observed. Other events did not appear to be related to the drug since the reporting incidence was equal in active drug and placebo groups. No differences in side effects were found related to gender or weight.

PUBLICATIONS: Marino MT, Schuster BG, Brueckner RP, Lin E, Kamainskis A, Lasseter R. Population pharmacokinetics and pharmacodynamics of pyridostigmine bromide for prophylaxis against nerve agents in humans. J Clin Pharmacol, 38(3):227-35, 1998

Title: Forward Deployable Diagnostics for Infectious Diseases

Project #: DoD-12

Agency: DoD

Study Location: USAMRIID, Ft. Detrick

Project Status: Ongoing

Research Type: Development

P.I.: LTC E. Jarboe, DVM

Research Focus: Diagnosis

Start Date (CY): 1993

Est. Completion (CY): 2002

OVERALL PROJECT OBJECTIVE: Develop a series of simple diagnostic assays suitable for forward deployed preventive medicine teams, Area Medical, and Forward Laboratories.

SPECIFIC AIMS: Develop rapid and simple diagnostic tests to permit the identification of the causes of infectious outbreaks before they reach the epidemic levels. Serological assays are being developed for typhus fever, dysenteries caused by shigella and other bacteria, and leptospirosis. Tests using polymerase chain reaction (PCR) technology are being explored for diagnosing Dengue fever. Fluorescent assays for malaria and other parasitic diseases are under investigation. Hand-held chromatographic immunoassays are being developed for Dengue fever. Future applications include campylobacter, malaria, and arboviral and rickettsial disease agents.

Dipstick technology is being developed for Dengue fever as well as rickettsial disease agents responsible for scrub and endemic typhus, and spotted fever group diseases.

METHODOLOGY: Technologies used in this research program center on hand-held chromatographic immunoassays, electrochemiluminescence (ECL) detection, PCR assays, and dip-stick immunoassays.

EXPECTED PRODUCTS (MILESTONES): By FY02, transition to advanced development of a portable device capable of detecting and identifying evidence of infection by militarily important pathogens in diverse clinical specimens such as respiratory secretions (influenza, adenovirus), stool (Shigella, Campylobacter, Enterotoxigenic E. coli, Salmonella, Norwalk virus), blood (Dengue, Lassa, malaria, meningococcus), urine (Leptospirosis), cerebrospinal fluid (Japanese encephalitis, Tick-borne encephalitis), and in skin lesions (Leishmaniasis).

STATUS/RESULTS TO DATE: Incorporated primers which amplify the 'rfc' gene which encodes O-antigen polymerases of Shigella species into a multiplex polymerase-chain reaction (PCR) with the Campylobacter jejuni 'ceuE' gene, necessary for development of a sensitive, specific forward deployable diagnostic device capable of detecting multiple infectious pathogens of military interest. Produced both recombinant and native Hantavirus nucleocapsid antigens for use in development and testing of diagnostic assays for Hantavirus infection. Produced native and recombinant Dengue antigens and attached them to platforms capable of supporting field diagnostic assay requirements. Identified field sites in Peru capable of supporting analysis of malaria diagnostic tests. Increased temperature stability of viral diagnostic reagents by lyophilizing them, demonstrating a technology that will be required in order to meet milestone exit criteria for all diagnostic devices. Completed initial field evaluation of a commercially produced hand-held Dengue diagnostic assay in Concept Evaluation Phase (CEP). Tests on 80 documented positive and 17 documented negative patients in Indonesia showed that the hand-held assay exceeded sensitivity and specificity of the current reference laboratory diagnostic methods. Completed initial field CEP evaluation of one commercially produced hand-held malaria diagnostic assay on patients in Indonesia with initial results showing very high sensitivity. Completed a limited field trial in Peru to evaluate the sensitivity of two commercially produced hand-held malaria diagnostic assays in detecting P. vivax. Enrolled over 3,000 volunteers in Peru and Thailand and completed 80% of testing in an expanded comparison of the malarial diagnostic assay candidates to demonstrate performance characteristics on different types of malaria worldwide. This comparison is critical to ensure that we will be able to detect malaria infection in our service members despite parasite variability throughout the world.

PUBLICATIONS: Moss, DW, Rossi CA, Mihalic KA, Ludwig GV, Phillips S, Henchal EA. Development of a rapid lateral flow assay for diagnosis of hemorrhagic fever with renal syndrome. 47th Annual Meeting of the American Society of Tropical Medicine and Hygiene, OCT 18-22, 1998, San Juan, Puerto Rico. Abstract, 1998.

Kelly DJ, Chan CT, Paxton H, Thompson K, Howard R, Dasch GA. Comparative evaluation of a commercial enzyme immunoassay for the detection of human antibody to Rickettsia typhi. Clinical & Diag Lab Immun, 2(3):356-60, 1995.

Weddle JR, Chan TC, Thompson K, Paxton H, Kelly DJ, Dasch GA, Strickman D. Effectiveness of a dot-blot immunoassay of anti-Rickettsia tsutsugamushi antibodies for serologic analysis of scrub typhus. Am J Tropical Med & Hyg, 53(1):43-6, 1995.

Title: Effects of Persian Gulf War Service on Military Working Dogs

Project #: DoD-13

Agency: DoD

Study Location: AFIP, Wash. DC

Project Status: Ongoing

Research Type: Epidemiology Research

P.I.: LTC Dale Dunn. DVM

Research Focus: Environmental Toxicology

Start Date (CY): 1994

Est. Completion (CY): 2001

OVERALL PROJECT OBJECTIVE: The possibility of exposure to environmental factors and endemic diseases exist for the population of military working dogs (MWDs) that deployed to the Persian Gulf (PG) theater of war. The question to be answered is: In the final analysis, what are the differences in diagnoses between the PG MWD cohort and a matched (on the basis of age, sex and breed) comparison group which never deployed to Southwest Asia (SWA).

SPECIFIC AIMS: The (null) hypotheses to be tested is that in the final analysis there will be no differences in the diagnoses between the PG MWD cohort and the comparison group which never deployed to SWA. Should this hypothesis not be supported: 1) the possibility exists that differences in diagnoses between the two groups may be the result of deployment to SWA; and 2) dates of deployment and location in theater will be compared among the PG MWDs, and conceivably to those of PG veterans.

METHODOLOGY: The PG MWD cohort was identified after the cessation of hostilities and subsequent redeployment. The inclusive deployment dates for this population are 1 August 1990 to 31 December 1991. MWDs receive semi-annual physical examinations throughout their active duty lives, which include clinical evaluations and routine panels of hematologic, serologic, and blood chemical analyses. The results of these tests are recorded in the animal's medical record. Those MWDs that are euthanized will have peripheral blood samples collected prior to euthanasia for the above tests. When natural death occurs, the most recent blood test will be used. Test results are to be included in the dog's permanent medical record. Necropsies are performed in accordance with a standard protocol contained in TB Med 283. Medical records from all deceased MWDs in the Department of Defense are archived at the DODMWDVS, Lackland AFB, Texas.

Based on the assumptions of a condition with 10% prevalence in the population, looking for a relative risk of 2.5 in the exposed group, setting the alpha level at 0.05 and the beta level at 0.2, a minimum of 112 animals of each group must be included in the study. Therefore, the medical and training records of those 118 MWDs which deployed to the Persian Gulf, and 472 non-deployed MWDs matched four to one based on age, gender and breed, will be abstracted during the study period for the following variables: animal identification; age at death; date of death; breed; gender; location during the time frame 1 August 1990 to 31 December 1991; duration of deployment; neurologic illness; orthopedic illness; dermatological illness; gastrointestinal illness; infectious diseases; parasitism; neoplasms; behavioral changes after 1 August 1990; pathologic diagnoses of biopsy specimens and pathologic diagnoses of autopsy specimens. These data will be electronically stored in a database for statistical analysis using the SPSSR analysis program. Those Persian Gulf deployed MWDs still living, and age, gender, breed matched non-deployed control MWDs will be transported to the DODMWDVS, Lackland AFB when the responsible Veterinary Corps officer has determined the animal is no longer physically fit for duty and in need of humane euthanasia. Upon arrival, the medical record will be screened to determine the cohort of assignment. The MWD will receive a complete physical exam, to include the following: CBC; serum chemistry panel; serum acetylcholinesterase activity levels; urinalysis; fecal exam for parasites; canine thyroid hormone measurements (T4, cTSH); electrocardiography; radiography; a neurologic examination and a behavioral assessment.

The MWD will be anesthetized according to a standard approved protocol. Radiographs of elbows, stifles, coxofemoral joints and spine will be obtained if not present in the record. Electromyograms and nerve conduction studies will then be conducted on the anesthetized dog, to determine neuromuscular function.

Euthanasia of the dog will be completed with a standard approved injectable euthanasia agent (Beuthanasia) and immediately necropsied in accordance with the TB Med 283. At necropsy, gross changes are described and an extensive set of tissues collected and forwarded to the Department of Veterinary Pathology, Armed Forces Institute of Pathology, Washington, DC. Muscle biopsies of the biceps femoris and triceps brachii; and nerve biopsies of the tibial and radial nerves will be for analysis at Auburn University. Additionally, 6 gram samples of liver, kidney, lung, brain and fat will be collected for ultra low temperature freezing and stored at the

AFIP or DoD Veterinary Laboratory until toxicological procedures may be performed, if indicated. Formalin fixed tissues will be processed for histopathologic examination resulting in a detailed final pathology diagnostic case report consisting of a list of pathologic findings and an interpretation of these findings. Remaining wet tissues, paraffin blocks, microslides and case folder materials will be archived. The pathology report will be forwarded to the DODMWDVS, Lackland AFB, TX for inclusion in the MWD's medical record.

All clinical and pathological information collected during the final examination procedures will be electronically stored in a database for statistical analysis using the SPSSR statistical program. Upon completion of initial data and records collection data, a multivariate analysis of collected variables will be accomplished to determine the effects of age, gender and breed on those conditions commonly occurring in the entire population of MWDs. Odds ratios and ninety-five percent confidence intervals will be calculated on all conditions occurring more frequently in one cohort to determine the effects of the exposure status on those conditions. Fishers's exact p values will be calculated to determine statistical significance of any conditions occurring more frequently in one cohort.

EXPECTED PRODUCTS (MILESTONES): See Objectives.

STATUS/RESULTS TO DATE: Ongoing. We have identified one hundred eighteen MWDs that deployed to various locations in the PG Theater in support of operations within the inclusive dates listed above.

Approximately 13% (15 animals) of the study group remain alive; most of these MWDs are still on active duty (12 animals), while a small number have been removed from active duty and relocated to DoD Military Working Dog Veterinary Services (DoDMWDVS), Lackland AFB (3 animals). The AFIP funded a pilot protocol enabling the early implementation of a data collection system that includes pathologic (including surgical and post-mortem morphologic changes), demographic, and clinical findings from initially identified PG MWD cohort and matched comparison MWDs. This database is now operational for all but the clinical data section. Over 200 modifications and improvements were made in FY98. The clinical data portion is currently under construction.

The collection and analysis of epidemiological data is the primary responsibility of the US Army Veterinary Corps Officer currently stationed at the DODMWDVS, Lackland AFB, TX. The retrospective cohort of PG-deployed dogs and non-deployed control and pathology data entered into the database. Clinical evaluation and data collection remaining dogs must be entered into the database and analyzed with the rest of the data in order to draw conclusions and compare the two cohorts.

Progress on this study was slowed between early December and late April by an erroneous press report released by the American Humane Association (AHA) that stated the DoD was killing dogs that served in the Persian Gulf war. AHA also started a mailing campaign and filed two Freedom of Information Act Requests (FOIA). Investigators, public affairs officers, and legal counsels at AFIP and DoDMWDVS spent considerable time on this issue answering press and congressional inquiries. A face to face meeting with AHA at Lackland AFB finally resolved AHAs concerns and resulted in the withdrawal of their FOIAs.

Necropsy tissues collected from 33 military working dogs were lost in a construction accident at Lackland AFB in early FY'98. Some of these dogs were PG-deployed dogs. The cohort totals will be reduced accordingly.

Major capital improvements and equipment required this fiscal year included construction of a state-of-the-art necropsy facility and purchase of an electroencephalogram/ electromyogram, a microscope, ultra-low freezer (DoDMWDVS), two vented storage cabinets (DoDMWDVS and AFIP), two tissue grossing stations (AFIP) and two Stryker saws (AFIP and 51st Med Det).

PUBLICATIONS: none to

Agency: DoD
Study Location: WRAIR, Wash. DC
Project Status: Complete
Research Type: Epidemiology Research
P.I.: James Writer, MPH
Research Focus: Symptoms/General Health
Start Date (CY): 1993
Est. Completion (CY): 1997

OVERALL PROJECT OBJECTIVE: Determine the presence of unique characteristics (demographics, aptitude test scores, self-reported health behaviors, past hospitalizations) of Army personnel enrolled in the VA Registry of Persian Gulf veterans.

SPECIFIC AIMS: There were two specific aims of this hypothesis-generating study. The first was to investigate logistical, epidemiological, and statistical methodologies for obtaining, combining, and analyzing records. These techniques will be needed for future studies on reported post-deployment illnesses in soldiers who served in the Gulf War, and will also be applicable in evaluating the health effects of future deployments. The second aim was to determine which characteristics (to include demographics, past medical history, and health risk behaviors) of soldiers who have been enrolled in the VA Gulf War registry differs from those who have not enrolled. Ultimately, this information can be used to identify soldiers at a high-risk of reporting post deployment medical problems. These soldiers may be targeted for interventions before or after their deployment to reduce the risk adverse health effects attributable to service in a war zone.

METHODOLOGY: Using case-control methodology the investigators studied the association between Gulf War veterans' various demographic, medical, or health behavior characteristics and their probability of enrolling in the Gulf War registry. The study population consisted of individuals on the VA database and controls from the Defense Manpower Data Center's (DMDC) roster of Gulf War participants. Characteristics evaluated included demographic factors, recruit applicant aptitude test scores, pre-deployment self-reported health behaviors, and pre-deployment health care utilization as are represented by hospitalizations in military hospitals. These data are available in already existing Army or DoD databases, however, all data may not be available for all study subjects.

EXPECTED PRODUCTS (MILESTONES): A data base of US Army Gulf War veterans, which includes health indicators and demographic data from the pre, during, and post deployment phases of their military careers was generated. The study determined if existing data sources can be used to predict a soldier's likelihood of having significant post-deployment adverse health outcomes or a high level of concern about potential health effects of the deployment.

STATUS/RESULTS TO DATE: Preliminary data analysis presented at the Armed Forces Epidemiology Board's December 1996 meeting. Manuscript is being prepared.

PUBLICATIONS: none to

Title: Comparative Mortality Among US Military Personnel Worldwide During Operations Desert Shield and Desert Storm

Project #: DoD-15

Agency: DoD

Study Location: WRAIR, Wash. DC

Project Status: Complete

Research Type: Epidemiology Research

P.I.: James Writer, MPH

Research Focus: Mortality

Start Date (CY): 1994

Est. Completion (CY): 1995

OVERALL PROJECT OBJECTIVE: Determine if military personnel deployed to Operations Desert Shield/Desert Storm have higher death rates than military personnel who did not deplv.

SPECIFIC AIMS: Characterize the disease and non-battle injury (DNBI) mortality experience of US military personnel during ODS/DS. Determine whether US military personnel deployed to Southwest Asia (SWA) had a higher rate of death than US military personnel who did not deploy.

METHODOLOGY: Casualty reports were used to calculate cause-specific mortality rates among all personnel on active duty in the US Armed Forces (including Reserve and National Guard) over a one-year interval that included the Persian Gulf War. Death rates among active duty personnel deployed to SWA at any time during this period were compared to rates for all military personnel on active duty but not deployed to SWA. Each death was characterized as to have been most likely caused by disease or trauma. Trauma deaths were additionally classified as due to unintentional (accidental) injury, battle injury, self-inflicted injury, or homicide.

EXPECTED PRODUCTS (MILESTONES): Determination of whether US military personnel deployed to SWA had a higher death rate than non-deployed military personnel.

STATUS/RESULTS TO DATE: Except for hostile action deaths, the overall mortality experience among US military personnel who deployed did not significantly differ from that observed among US military personnel who did not deploy to SWA. No evidence of clusters of unexpected deaths were found, except for two accidents. The number and circumstances of nonbattle deaths among Persian Gulf troops were typical for the U.S. military population.

PUBLICATIONS: Walter Reed army Institute of Research Report. Comparative Mortality Among US Military Personnel Worldwide During Operations Desert Shield and Desert Storm. January 1995.

Writer JV, DeFraites RF, Brundage JF. Comparative mortality among US military personnel in the Persian Gulf region and worldwide during Operations Desert Shield and Desert Storm. JAMA, 275(2):118-121, 1996.

Title: Kuwait Oil Fire Health Risk Assessment

Project #: DoD-16

Agency: DoD

Study Location: CHPPM, Aberdeen MD

Project Status: Complete

Research Type: Development

P.I.: Jack Heller, Ph.D.

Research Focus: Environmental Toxicology

Start Date (CY): 1991

Est. Completion (CY): 1994

OVERALL PROJECT OBJECTIVE: To characterize both the carcinogenic and noncarcinogenic health risks to DOD troops and civilian employees exposed to the environment affected by the oil fires during and after Operation Desert Storm.

SPECIFIC AIMS: 1. Environmental Monitoring - The environmental monitoring study attempted to characterize the concentration of pollutants that DOD personnel were exposed to during their deployment in the Gulf region. Air monitoring and soil sampling data were used to calculate exposure point concentrations for individual Health Risk Assessments in the final report. 2. Industrial Hygiene Sampling Study - The industrial hygiene air survey monitored and characterized occupational exposures of DOD personnel who had potential high risk exposure to oil fire emissions. The focus was on individuals working outdoors and on worst-case situations within the oil fields next to Kuwait City. 3. Biological Surveillance Initiative - The Biological Surveillance Initiative was conducted to refine and corroborate the results from the Health Risk Assessment. This was accomplished by a collection of objective biological measurements of exposure and effect in real-time, and by establishment of any observable biologic effect or marker of exposure to oil fire pollutants in a cohort of U.S. soldiers.

METHODOLOGY: 1. Sampling and Analysis - Sampling and analytical methodologies for ambient air, soil, industrial hygiene air, biologic samples, and quality assurance procedures are

detailed in the final report. 2. Risk Assessment - The Methodology used for this Health Risk Assessment was the EPA guidance for the Comprehensive Environmental Response, Compensation and Liability Act sites (CERCLA), also known as "Superfund" sites. The Methodology did not determine an individual's health outcome or include the use of reported health effects data. The components of the risk assessment process included data collection and evaluation, exposure assessment, toxicity assessment, and risk characterization and uncertainty analysis.

EXPECTED PRODUCTS (MILESTONES): Reports

STATUS/RESULTS TO DATE: Completed.

The potential for significant long-term adverse health effects for the exposed DOD troop or civilian employee populations is minimal. The total predicted excess carcinogenic risk both in Kuwait and in Saudi Arabia did not exceed 3 excess cancers per 1,000,000 population exposed. The predicted carcinogenic risk levels are well within the EPA range of acceptable excess carcinogenic risks. The majority (>99%) of noncarcinogenic risk at all monitoring sites is predicted to be from the inhalation of volatile organic compounds and is assessed as low. The results of the Biological Surveillance Initiative support the conclusion that noncarcinogenic risk levels indicate minimal potential adverse health effects.

As of January 1998, the DoD Persian Gulf registry unit movement database contains location data for 3,198 Army units, 248 Navy sea and ground units, and 1,021 Marine Corps units. A location code is annotated for Air Force personnel in the DoD Persian Gulf personnel database. The location code replaces the unit identification code (UIC) for tracking purposes. To date, 84,925 Air Force personnel contain location codes.

The U.S. Armed Services Center for Research of Unit Records (CRUR) reviewed approximately 6 million pages of documents and has conducted fourteen S-3/G-3 conferences. The S-3/G-3 conferences consist of former Operation Desert Shield / Desert Storm operations officers (at the brigade and division levels) meeting at the CRUR to review existing unit movement and location records. From the reviews, enhancements were made to the database to include data gap-fills, unit additions, and unit movement location data down to the company and detachment level. The database currently contains approximately 700,000 grid coordinates, latitudes/longitudes and names of base camp locations. It is important to note that the database will continue to increase in size when additional records are found. The resolution of the database will further increase because additional S-3/G-3 conferences were held in 1998.

PUBLICATIONS: US Army Environmental Hygiene Agency, Final Report. Kuwait Oil Fire Health Risk Assessment. No. 39-26-L192-91, 18 February 1994.

Title: Retrospective Studies Involving Military Use of Pyridostigmine as a Pretreatment for Nerve Agent Poisoning

Project #: DoD-17

Agency: DoD

Study Location: WRAIR, Wash. DC

Project Status: Complete

Research Type: Epidemiology Research

P.I.: LTC Mary Burman

Research Focus: Pyridostigmine Bromide

Start Date (CY): 1991

Est. Completion (CY): 1992

OVERALL PROJECT OBJECTIVE: To obtain safety data for New Drug Application with the Food and Drug Administration (FDA).

SPECIFIC AIMS: To perform retrospective evaluation of effects of pyridostigmine use in the Persian Gulf.

METHODOLOGY: Retrospective evaluation.

EXPECTED PRODUCTS (MILESTONES): Reports.

STATUS/RESULTS TO DATE: Complete.

Three surveys were completed, reports prepared, and forwarded to the FDA. Results indicated that pyridostigmine continues to be safe. Side effects reported were primarily gastrointestinal in nature. Headaches were also reported by the respondents.

PUBLICATIONS: Three Survey Reports consolidated Into an Amendment to the FDA Investigational New Drug Application

Title: Kuwait Oil Fires Troop Exposure Assessment Model (TEAM)

Project #: DoD-18

Agency: DoD

Study Location: CHPPM, Aberdeen MD

Project Status: Ongoing

Research Type: Development

P.I.: Jack Heller, Ph.D.

Research Focus: Environmental Toxicology

Start Date (CY): 1993

Est. Completion (CY): 2006

OVERALL PROJECT OBJECTIVE: To respond to Public Law 102-190 (Section 734: Troop Registry from exposure to oil well fires in Operation Desert Storm) by characterizing the potential carcinogenic and non-carcinogenic health risks to U.S. military personnel exposed to the environment affected by the oil well fires during and after Operation Desert Storm.

SPECIFIC AIMS: The site-specific Kuwait Oil Fire Health Risk Assessment addressed the risks to DOD troops located at the eight fixed air/soil sampling sites where actual environmental data were collected and analyzed. This study will incorporate actual site data with the modeled air concentrations, exposure data, and the Troop Location Registry data, to determine the risk to U.S. military personnel that were not located at fixed sampling sites for their entry tour of duty and for periods of time when no sampling occurred.

METHODOLOGY: Using a geographic information system (GIS) that is capable of mapping troop location and movement over time, in conjunction with the troop movement database, the locations of all US military personnel are being mapped during Operation Desert Storm. Once troop locations have been determined and mapped, the GIS determines troop exposure to oil fire pollution using satellite images, modeled plume boundaries, oil field crude composition data, oil field emission rates, modeled pollutant concentrations, and actual field data. When troop location and exposure have been determined, the potential health risks resulting from the exposure will be determined.

EXPECTED PRODUCTS (MILESTONES): The outcomes expected from this study will be the determination of exposure and potential health risks associated with oil fire pollution for U.S. military personnel participating in Operation Desert Storm.

STATUS/RESULTS TO DATE: This project is ongoing. A majority of the data has been entered into the system. Both satellite plume and modeled plume boundaries (over 270 days, for the February – October 1991 timeframe) have been digitized. Pollutant-specific oil fire emission factors have been derived and integrated into the TEAM; updates on these are on going. Troop unit identification code location/movement data for the Navy, Marine Corps, and Air Force, and Army (over 500,000 records) has been entered into the TEAM database. We are continually updating troop unit movement data and will integrate into the TEAM as it is received. In addition, the Desert Storm personnel rosters (696,693 individuals) are in the TEAM database along with the DoD's Comprehensive Clinical Evaluation Program (CCEP) database. We are currently updating the toxicological data (from USEPA IRIS and HEAST databases) and exposure factor tables of the TEAM database. In all, over 2.25 million records have been entered into the TEAM database. In FY'98 the TEAM has calculated the exposure and risk for all troop units exposed to

oil fire smoke in the Persian Gulf. The next step will be to determine the exposure and risk for the individuals in each unit.

PUBLICATIONS: US Army Environmental Hygiene Agency, Final Report. Kuwait Oil Fire Health Risk Assessment. No. 39-26-L192-91, 18 Feb 1994. This report describes the eight fixed air/soil sampling sites study and presents the preliminary results of the KOFTEAM pilot project.

Title: Persian Gulf Veterans Health Tracking System

Project #: DoD-19

Agency: DoD

Study Location: CHPPM, Aberdeen MD

Project Status: Ongoing

Research Type: Development

P.I.: Jack Heller, Ph.D.

Research Focus: Environmental Toxicology

Start Date (CY): 1996

Est. Completion (CY): 2003

OVERALL PROJECT OBJECTIVE: To respond to Public Law 102-585 (Section 702: Scientific research using Troop Health Registry) by characterizing all potential exposures (e.g., oil fires, vaccines, chemical /biological warfare agents, pesticides, pyridostigmine bromide, etc) to U.S. military personnel participating in Operation Desert Storm, and to assess the potential health consequences of those exposures.

SPECIFIC AIMS: The site-specific Kuwait Oil Fire Health Risk Assessment and the Troop Exposure Assessment Model (TEAM) address the potential risks to U.S. military personnel from potential exposure to airborne contaminants from oil well fires. This study attempts to characterize other potential exposures experienced by U.S. military personnel during Operation Desert Storm and to assess the potential health risks/consequences of those potential exposures.

METHODOLOGY: The Persian Gulf Veterans Health Tracking System uses a geographical information system (GIS) to assess potential exposures and medical outcomes for U.S. military personnel who participated in Operation Desert Storm. Once troop locations are determined and mapped (from the Troop Location Registry data), the GIS can help determine potential troop exposure to any number of different chemicals, environmental threats, or other materials. When troop locations and potential exposures have been determined, the potential health risks resulting from the potential exposures can be determined. In addition to examining potential exposure, databases on medical outcomes (e.g., Comprehensive Clinical Evaluation Program (CCEP), the Veterans Administration Mortality Study, etc.) can be evaluated by studying potential exposures, locations, movements, and relationships of the troops in these databases for epidemiological significance.

EXPECTED PRODUCTS (MILESTONES): Characterization of the potential exposures and health risks/outcomes associated with service by U.S. military personnel in Operation Desert Storm.

STATUS/RESULTS TO DATE: Since the March 1996 updates, we have received official confirmation from MEDCOM to proceed with this project. From July 1995 to April 1996, the CHPPM had developed a conceptual protocol for this project. This conceptual protocol integrates all potential environmental exposure and medical outcome databases for Operation Desert Storm. During this period of time, we had also reassessed the exposure databases which will be integrated into the Persian Gulf Veterans' Health Tracking System GIS. This reassessment was based on several requests from the Persian Gulf War Veterans' Illness Investigative Team (now the Office of the Special Assistant for Gulf War Illnesses [OSAOWI]) and the Assistant Secretary of Defense for Health Affairs, to concentrate on those environmental exposures deemed most critical to Gulf War Veterans' illnesses. These environmental databases include low-level

chemical and biological exposure (from coalition bombings and munitions storage bunker detonations) and pesticide usage and their possible interaction. Other environmental exposure data sets/databases include regional particulate matter concentrations for the Operation Desert Storm theater of operations; regional depleted uranium (DU) air concentrations for the Operation Desert Storm theater of operations and botulinum toxoid and anthrax vaccinations. In addition, taskings were received which required integration of the CCEP database into the GIS from which medical outcome and exposure relationships can be investigated.

To date, this project is ongoing. We have integrated the CCEP database into the GIS. Space and time analyses are being performed on the possible low-level chemical agent exposure from the munitions storage bunker detonations at Khamisiyah and air war attacks on other sites. The soldiers and Units identified in the CCEP, the U.S. Armed Services Center for Research of Unit Records Troop Unit Movement Database, and the Defense Manpower Data Center Operation Desert Storm personnel roster are being mapped with respect to their proximity to these sites. Exposure analyses are being performed with dispersion modeling results from the Central Intelligence Agency, Defense Threat Reduction Agency, and Naval Surface Warfare Center. We are planning to integrate the chemical detection network data into the O18 for additional low-level chemical agent analyses. In addition, other environmental exposure data sets/databases to include scud sites, tent heaters, microwaves, and dust suppression are being collected and assessed. This year additional exposure data on chemical agents, DU, particulate matter, and oil well fires was added to the system databases, in addition to improved troop unit movement data.

PUBLICATIONS: none to date

Title: A Statistical Study Correlating the Reported Cases of Gulf War Syndrome to Battlefield Locations of Afflicted US Army Personnel During the Iraq-Kuwait War; Part 1: Method to Related Troop Deployment and the Reported Cases of Gulf War Syndrome and Probable Incidence of Maladies Defined by ICD-9-CM

Project #: DoD-20

Agency: DoD

Study Location: USAR Lab, APG, MD

Project Status: Complete

Research Type: Epidemiology Research

P.I.: Dr. Gluckman

Research Focus: Symptoms/General Health

Start Date (CY): 1994

Est. Completion (CY): 1995

OVERALL PROJECT OBJECTIVE: To devise a procedure for counting the collections of symptoms and diagnoses of veterans' illnesses and relating them to the U.S. military grid system locations in the Kuwait-Iraq-Saudi Arabian theater of operations.

SPECIFIC AIMS: To identify the geographic locations by date of the Army personnel who served in the Gulf War and whose names are recorded in the VA register as experiencing severe health problems. The units to which these personnel were assigned are tracked across the Iraq-Kuwait region, in order to determine whether epidemiological commonalties can be detected based upon location at certain dates.

METHODOLOGY: VA malady codes of afflicted personnel related to unit identification codes were identified. Malady information was regrouped and coded into crisp and fuzzy numbers. For the Part 1, only crisp numbered malady information was used. The DOD Register was used to identify geographic coordinate locations of Army units. A mathematical algorithm was developed to compare geographic location to maladies and possible cause(s) of each of the maladies. The algorithm is based on the ratios of the number of combatants reporting illnesses (in any grid) to the total number of combatants (occupying the grid) as discrete probabilities. These are considered to be the probabilities of occurrence of a health condition for the total time period that a military unit occupies a grid. A production model needs to be completed to compute statistical measures that are concerned with Gulf War veterans' illnesses.

EXPECTED PRODUCTS (MILESTONES): A mathematical procedure to count the collections of symptoms and diagnoses over the various map grids of the region.

STATUS/RESULTS TO DATE: Completed.

PUBLICATIONS: Army Research Laboratory Technical Report ARL-TR-800, July 1995

Title: Study of Variability In Pyridostigmine Inhibition of Blood Cholinesterases in Healthy Adults and Individuals With Symptoms Following Participation in Operation Desert Storm

Project #: DoD-21

Agency: DoD

Study Location: WRAIR, Wash. DC

Project Status: Complete

Research Type: Epidemiology Research

P.I.: Bhupendra Doctor, Ph.D.

Research Focus: Pyridostigmine Bromide, Prevention

Start Date (CY): 1995

Est. Completion (CY): 1997

OVERALL PROJECT OBJECTIVE: Assess individual differences in blood cholinesterase regulation and response to pyridostigmine.

SPECIFIC AIMS: Determine if individuals have different sensitivities to inhibition of blood cholinesterases by pyridostigmine and/or differences in the rate of spontaneous reactivation of cholinesterases after pyridostigmine exposure. Determine if there is a difference in the cholinesterases of Gulf War veterans who took pyridostigmine and seen at the Gulf War Health Center compared to age and sex matched controls.

METHODOLOGY: Blood (in EDTA) is obtained from Gulf War veterans seen at the Gulf War Health Center at Walter Reed Army Medical Center and from age and sex matched controls. Blood samples were assigned code numbers when drawn in the Gulf War Health Center, and the code was broken for the first 40 samples only after results from the data analysis were complete. Estimated spontaneous reactivation times after in vitro pyridostigmine inhibition, red cell acetylcholinesterase activity, and plasma butyrylcholinesterase activity were compared in blood samples from 20 veterans of Operation Desert Storm and 20 control subjects, matched for sex and age. Determination of plasma butyrylcholinesterase phenotypes was done at the Eppley Institute, University of Nebraska, Omaha, NE.

EXPECTED PRODUCTS (MILESTONES): Complete initial studies of the first 40 volunteers by FY96; and from 100 volunteers by FY97.

STATUS/RESULTS TO DATE: In the initial study of 40 volunteers, 18 of the 20 veterans in the study (90%) presented to the WRAMC Comprehensive Clinical Evaluation Program with post-war symptoms. All Gulf War veterans indicated they had taken pyridostigmine as a pretreatment drug for nerve agent exposure; 15 of 20 recalled having side effects after taking pyridostigmine. Mean red cell lysate acetylcholinesterase for all subjects was 5.7 U/ml +/- 0.7; mean plasma butyrylcholinesterase was 4.9 U/ml +/- 1.1. Mean spontaneous reactivation time (t-1/2 at 2.5 microM pyridostigmine) for all subjects was 42.6 min +/- 5.4; mean for veterans was 43.2 +/- 6.2; mean for controls was 42.1 min +/- 4.6. Statistical analysis of reactivation times (repeated measures ANOVA) revealed no statistically significant differences between controls and veterans. However, significant differences in reactivation times (p=0.01) were observed between males and females across both groups. Differences in cholinesterase activities between controls and veterans were not statistically significant, but a differences between sexes was again significantly different. The occurrence of the UU (homozygous, usual, wild type) and UA (heterozygous, usual, atypical) phenotypes of butyrylcholinesterase in this study population [95% (38 of 40) and 5% (2 of 40), respectively] is close to the expected frequency, 96% and 4%. All Gulf War veterans in the study had the homozygous, usual, wild-type (UU) allele. Symptoms exhibited by these individuals do not appear to be related to either altered ability of red cell acetylcholinesterase to be inhibited by pyridostigmine, to extended spontaneous reactivation time after pyridostigmine exposure, or to a genetic mutation in the butyrylcholinesterase phenotype. In the study of the remaining 60

volunteers, another 40 samples have been collected to date. The coding of these samples will not be examined until the remaining 20 blood samples have been analyzed.

PUBLICATIONS: In vitro pyridostigmine inhibition of red cell acetylcholinesterase: a comparison in Gulf war veterans and normal controls. 1996 Medical Defense Bioscience Review, May 1996, 1254-61. Baltimore, Maryland. Proceedings in press.
A comparison of blood cholinesterases, in vitro pyridostigmine inhibition, and butyrylcholinesterase phenotypes in Gulf War veterans and controls. The Second Chemical and Biological Medical Treatment Symposium, July 1996, 359-64. Spiez, Switzerland

Title: Chronic Organophosphorous Exposure and Cognition

Project #: DoD-22

Agency: DoD

Study Location: Med Coll of GA Res Inst., Augusta

Project Status: Complete

Research Type: Mechanistic

P.I.: Jerry J. Buccafusco, Ph.D.

Research Focus: Environmental Toxicology, Brain & Nervous System, Chemical Weapons

Start Date (CY): 1995

Est. Completion (CY): 1998

OVERALL PROJECT OBJECTIVE: This study will evaluate the effects of low-level sub-chronic exposure to an organophosphorous cholinesterase inhibitor on normal cognitive function in animal models. The long term goals are to identify the underlying mechanisms of organic brain damage related to environmental toxins and to develop novel treatment strategies to improve memory/cognitive performance in affected patients.

SPECIFIC AIMS: This study will address four main questions: 1. Does low-level sub-chronic exposure to an organophosphorous (OP) cholinesterase inhibitor produce learning or memory deficits in rats and non-human primates trained to perform various memory tasks; and will prior exposure to atropine sensitize animals to OP agents? 2. Is memory impairment to an OP agent associated with alterations in the presynaptic regulation of cholinergic neurons in relevant brain regions in rats; and/or is there an alteration in the ability of the brain adrenergic system to regulate presynaptic cholinergic function? 3. Is memory impairment to an OP agent associated with alterations in the postsynaptic regulation of cholinergic or adrenergic receptors? Can these alterations be reflected in the genes encoding the various muscarinic or nicotinic receptor subtypes? 4. Can new therapies (reversible cholinesterase inhibitors, nicotinic agonists, adrenergic agonists) which improve memory deficits in Alzheimer's patients also improve cognitive function in rats or monkeys with OP-induced memory impairment?

METHODOLOGY: 1) Groups of at least 12 rats each will be administered various doses of DFP once daily over 2 weeks. The status of brain cholinergic receptors and blood cholinesterase will be determined and 2 days - 3 weeks after the last dose of DFP they will be examined for performance in the Morris water maze. Monkeys will be well-trained in the delayed matching-to-sample (DMTS) task. The highest dose of diisopropylfluorophosphate (DFP) which does not elicit overt signs of anticholinesterase toxicity or which will not decrease DMTS performance will be employed in the study. 2) Rats will be administered a 14 day DFP regimen. Samples of regional brain tissue are taken on 4 occasions during treatment to determine the status of muscarinic, nicotinic and alpha-adrenergic receptor subtypes in several brain regions using quantitative receptor autoradiography. Small samples of tissue will be removed for quantitative RT-PCR. 3) The rats which have been evaluated in Protocol 1 for production of long-term decrements in DMTS performance will be employed. The drugs to be tested in this protocol will include compounds such as nicotinic receptor agonists that we have previously showed to produce improvement in memory-related task performance.

EXPECTED PRODUCTS (MILESTONES): This research will be dedicated to the elucidation of

the mechanism of permanent memory dysfunction produced by exposure to a relevant organophosphorous agent DFP in non-human primate and rat models. Also, we will evaluate the effects of reversing this memory impairment using classical and novel agents developed for patients with Alzheimer's disease.

STATUS/RESULTS TO DATE: Chronic, low-level exposure to acetylcholinesterase (Ache) inhibitor organophosphate (OP) insecticides or chemical warfare agents produces abnormalities in CNS acetylcholine (ACh) function, and in humans, may be associated with impaired cognitive function well after withdrawal from such exposure. The purpose of the present study was to identify the severity of impairment in spatial learning of rats and monkeys following protracted withdrawal from chronic, low-level exposure to the OP agent DFP. Assessment of spatial learning began either 1 - 17 days after completion of a 14 day DFP treatment regimen (50 - 500 mg/kg). During the 14 day treatment regimen, prior to withdrawal, spontaneous activity and olfactory behaviors were initially suppressed during DFP exposure, effects which became tolerant with repeated exposure to the 250 mg/kg dose regimen. Performance of a standard water maze task was impaired for up to 21 days after withdrawal from treatment with a 250 mg/kg dose of DFP. Performance of a standard water maze task was impaired for up to 21 days after withdrawal from treatment with a 250 mg/kg dose of DFP. Performance of a previously well-learned delayed matching task by monkeys was not affected by DFP regimens after withdrawal of the drug. Likewise, DFP selectively impaired the acquisition of the rodent spatial memory task in rats without impairing performance in animals pre-trained in the task. These results complement those showing that a similar DFP regimen did not alter memory-related task performance in rats working a previously well-learned delayed discrimination task. The DFP regimen induced a protracted decrease in the expression of nicotinic and muscarinic (M2) receptors in several brain regions. However, in certain regions of the hippocampus (e.g., CA1 or subiculum) the decreased expression of nicotinic receptors was still evident after 3 weeks of withdrawal from DFP. Levels of AChE activity also were slower to recover for this brain region. In general, cholinergic macromolecules that are located primarily at postsynaptic sites (nicotinic and M2 muscarine receptors) were affected to a greater extent, and for a longer time period than were those that are located primarily presynaptically (M1 muscarinic receptors and acetylcholinesterase). This decreased rate of cholinergic macromolecule recovery may be related to a reduced synthesis or transport of "postsynaptic" cholinergic macromolecules to nerve endings; and may underly the protracted impairment of working memory. DFP-induced spatial memory impairment was completely inhibited either by addition of pyridostigmine to the regimen, or by pre-testing (post-regimen) injection of nicotine. The latter effect was of particular importance because (1) it shows that it may be possible to treat memory disorders associated with long term exposure to OP agents, and (2) nicotine was shown to rapidly increase the expression of the mRNA that encodes the vesicular acetylcholine transporter. The transporter is a marker for cholinergic nerve terminals and is important for the synthesis and release of the neurotransmitter.

PUBLICATIONS: Gattu M, Boss K, Terry A, Buccafusco J. Reversal of Scopolamine-Induced Deficits in Navigational Memory Performance by the Seed Oil of *Celastrus paniculatus*, (57):4:793-799, 1997.

Prendergast M, Terry A, Buccafusco J. Chronic, low-level exposure to diisopropylfluorophosphate causes protracted impairment of spatial navigation learning. *Psychopharmacology*, 129:183-191, 1997.

Prendergast MA, Terry AV, Buccafusco JJ. Effects of chronic, low-level organophosphate exposure on delayed recall, discrimination, and spatial learning monkeys and rats. *Neurotoxicol Teratol*, 20: 115-122, 1998.

Prendergast MA, Buccafusco JJ. (-)Nicotine exposure increases mRNA encoding G3PDH and the vesicular acetylcholine transporter in vivo. *Neuro Reports* 9:1385-1389, 1998.

Buccafusco JJ, Prendergast MA, Pauly JR, Terry AV, Goldstein BD, Shuster LC. A rat model for Gulf War Illness-related selective memory impairment and the loss of hippocampal nicotinic receptors. *Soc Neurosci Abs*, 23:216, 1997

Title: Acute and Long-Term Impact of Deployment to Southwest Asia on the Physical and Mental Health of Soldiers and their Families

Project #: DoD-23

Agency: DoD

Study Location: WRAIR, Wash. DC

Project Status: Complete **Research Type:** Epidemiology Research

P.I.: MAJ John Stuart

Research Focus: Brain & Nervous System, Symptoms/General Health

Start Date (CY): 1993

Est. Completion (CY): 1994

OVERALL PROJECT OBJECTIVE: Determine the acute and long-term impact of deployment to SWA on the physical and mental health of soldiers and their families.

SPECIFIC AIMS: Determine the acute and long-term impact of deployment to SWA on the physical and mental health of soldiers organizational, and environmental factors within military units that produce psychiatric casualties. Identify the critical indicators resulting in increased risk and assess the long-term consequences of exposure to trauma. Develop recommendations on post-deployment interventions. Determine impacts of Army family support policies and programs on active duty and reserve component soldiers and their families.

METHODOLOGY: Interviews and surveys of soldiers and leaders participating in ODS were conducted several times prior to the ground war, immediately after the ground war, and at intervals following return home. Soldiers and their spouses were surveyed and interviewed in order to evaluate the social, psychological, organizational, and environmental factors within military units that produce psychiatric casualties, lead to dysfunctional behavior and decrements in military performance, generate psychosomatic illness and increased susceptibility to physical illness. The family-community research team performed field surveys of spouses of active duty and reserve component (USAR) and (ARNG) soldiers from units deployed to the Persian Gulf for ODS on deployment-related stressors and psychological symptoms, spouse support-seeking behavior, family perceptions of Army support and assistance, stress-coping responses, and family well-being during reunion with returning soldiers and in the aftermath of ODS. Surveys and interviews of selected Reserve and National Guard units and their families have been conducted during the period following ODS, while additional interviews and surveys were conducted with soldiers and families participating in Operation Just Cause and Sinai peacekeeping deployments.

EXPECTED PRODUCTS (MILESTONES): Develop recommendations affecting post-combat interventions. Determine the impact of Army family support policies and programs on both active duty and reserve component soldiers and families.

STATUS/RESULTS TO DATE: An assessment of psychological factors related to deployment stress and family separation during Operation Desert Shield/Storm was conducted with a stratified sample of married and single U.S. Army Individual Ready Reserve soldiers two years post ODS. Analyses were performed in 1994 with final report completed in spring of 1995 and submitted to Army Deputy Chief of Staff for Personnel. Deployment stress and adaptation was studied on a large sample of U.S. Army National Guard and U.S. Army Reserve Unit soldiers who activated and deployed for Operation Desert Shield/Storm. Physiological stress, distress, coping and factors related to current deployed effects of family separation were assessed. Physiological well-being was studied with respect to deployment location factors. Persian Gulf Region analyses were performed in summer of 1995 and resulted in a manuscript on the long term effects of Desert Storm on the Army Reserve and National Guard soldiers and their families.

PUBLICATIONS: Epidemiological considerations regarding the health and effectiveness of women in the armed forces. Technical Report, AD#ADA278202.

Combat service support survey results: A light infantry division and a mechanized infantry division. Technical Report AD#ADA278203.

The Long Term Effects of Operation Desert Storm on the Psychological Well-Being of US

Title: Epidemiological Studies Persian Gulf War Illnesses, PG Women's Health Linkage Study

Project #: DoD-30

Agency: DoD

Study Location: Klemm Analyses Group, Washington DC

Project Status: Ongoing

Research Type: Epidemiology Research

P.I.: Rebecca J. Klemm, Ph.D.

Research Focus: Symptoms/General Health, Reproductive Health

Start Date (CY): 1996

Est. Completion (CY): 2000

OVERALL PROJECT OBJECTIVE: Compare the incidence, prevalence, general health outcomes, and risks of women deployed in the Gulf War theater of operations with Gulf War era women who were not deployed to the Gulf.

SPECIFIC AIMS: The primary focus of this project is to link Persian Gulf exposures to subsequent physical and mental health outcomes.

METHODOLOGY: Self-reported survey information from a sample of 20,000 veterans will be linked to DoD tracking files and other mapping files and medical records.

EXPECTED PRODUCTS (MILESTONES): The survey will be fielded in early 1999. Medical record follow-up will begin during spring 1999. Data analysis will occur in the summer of 1999.

STATUS/RESULTS TO DATE: Data which is necessary to start the pilot study is being obtained from the Defense Manpower Data Center.

PUBLICATIONS: Klemm RJ. The Persian Gulf Health Linkage Study: a longitudinal study designed to assess short-term and long-term effects of Gulf War Service. American Public Health Association (APHA) annual meeting, November 1997

Title: Dysregulation of the Stress Response in the Persian Gulf Syndrome

Project #: DoD-31

Agency: DoD

Study Location: Georgetown Univ & VAMC Wash. DC

Project Status: Ongoing

Research Type: Clinical Research

P.I.: Daniel Clauw, M.D.

Research Focus: Brain & Nervous System, Symptoms/General Health, Diagnosis

Start Date (CY): 1996

Est. Completion (CY): 1999

OVERALL PROJECT OBJECTIVE: Test the hypothesis that Gulf War veterans who report persistent unexplained symptoms do not represent a discrete illness but instead fall within a continuum of stress-related syndromes commonly seen in the general U.S. population, including fibromyalgia (FM), chronic fatigue syndrome (CFS), multiple chemical sensitivity (MCS), and somatoform disorder (SD).

SPECIFIC AIMS: Specific hypotheses to be tested include: (1) Gulf War veterans with unexplained symptoms display centrally mediated disturbances in autonomic tone, and this leads to vasomotor instability and smooth muscle dysmotility, and symptoms such as irritable bowel syndromes and migraine headaches, (2) these same individuals display diffuse disturbances in nociception (pain threshold) that are partly responsible for many of the pain-related symptoms seen in this condition such as muscle and joint pain and persistent sore throat, (3) the same neuroendocrine changes seen in FM, CFS, and post-traumatic stress disorder (PTSD) which are

characterized by blunted responses in the hypothalamic-pituitary axis, are seen in these Gulf War veterans and may also be associated with fatigue symptoms.

METHODS: This study is a collaborative effort between Georgetown University Medical Center and the Washington D.C. Veterans Administration Medical Center (VAMC). The results of neurophysiological and neuropsychological testing will be compared between a group of Gulf War veterans and a group of individuals with FM/CFS. Participants are admitted to the Clinical Research Center at Georgetown for two days following their routine admission to the VAMC, and undergo a series of studies to evaluate physiologic and biochemical parameters. The physiologic studies measure qualitative and quantitative aspects of symptoms, including specialized testing of peripheral and visceral nociception, and smooth muscle motility. Biochemical analyses include evaluation of multiple indices of autonomic function, including neurohormone levels at baseline and after standardized stressors, and cerebral spinal fluid levels of neurotransmitters.

EXPECTED PRODUCTS (MILESTONES): A better understanding of common underlying pathophysiologic mechanisms in fibromyalgia, chronic fatigue syndrome, multiple chemical sensitivity and the undiagnosed illnesses of Gulf War veterans should lead to both more effective treatment of Gulf War veterans and produce effective strategies to avoid these problems in future deployments.

STATUS/RESULTS TO DATE: To date, 17 patients have been studied, as well as 38 FMS/CFS patients, and 27 healthy normal controls (these latter groups are for comparison). Preliminary data are available in many of the domains of testing; in other areas all samples will be run at the completion of the study because of concerns regarding inter-assay variability. Our findings in FM/CFS patients duplicated our pilot data and demonstrated that the FM/CFS group displayed increased peripheral and visceral pain sensitivity, a higher prevalence of esophageal (smooth muscle) dysmotility, and diminished sympathetic and (a trend towards) lower parasympathetic tone as measured by 24 hour heart rate variability (HRV). In general, the GWI patients demonstrated the same qualitative differences as patients with FM/CFS, although these were less pronounced, including intermediate levels of pain sensitivity, smooth muscle tone, and autonomic tone assessed by HRV.

PUBLICATIONS: Clauw D. The 'Gulf War Syndrome'; Implications for Rheumatologists. Journal of Clinical Rheumatology, 4(4);173-4, 1998.

Clauw D, Chrousos GP. Chronic Pain and Fatigue Syndromes: Overlapping Clinical and Neuroendocrine Features and Potential Pathogenic Mechanisms. Neurolmmunomodulation, 4;134-153, 1997.

Clauw D, King T, Barbey J, Benjamin S, Lyden A, Ambrose K, Groner K. Physiologic Abnormalities in Fibromyalgia, Chronic Fatigue Syndrome, and Gulf War Illness

Title: Neuropsychological Functioning in Persian Gulf Era Veterans

Project #: DoD-32

Agency: DoD

Study Location: VAMC Boston

Project Status: Ongoing

Research Type: Clinical Research

P.I.: Roberta White, Ph.D.

Research Focus: Brain & Nervous System, Diagnosis

Start Date (CY): 1996

Est. Completion (CY): 1999

OVERALL PROJECT OBJECTIVE: Persian Gulf War (PGW) veterans have reported a constellation of health symptoms that have been referred to popularly and in the media as "Gulf War Syndrome". The most commonly reported symptoms of the first 12,774 veterans who reported to their local VA (and registered with the Persian Gulf Registry) include, in order of frequency: excessive fatigue, skin rash, joint pain, headaches, disturbances of concentration and

memory. Several different possible causes of these symptoms have been suggested, including exposure to environmental hazards such as diesel fuel, oil fire smoke or pesticides, and biological or chemical warfare agents. Some of the symptoms reported overlap with those of post-traumatic stress disorder (PTSD), multiple chemical sensitivity (MCS), or chronic fatigue syndrome (CFS), three disorders of unknown mechanism that have been seen in other treatment seeking populations. Both exposure to neurotoxicants and the three disorders noted above are known to be capable of producing cognitive impairments. Neuropsychological tests of known validity and sensitivity have been used to document these impairments. The proposed study evaluates the neuropsychological functioning of PGW-era veterans who are seeking treatment or diagnostic evaluation for any type of health or adjustment complaint.

SPECIFIC AIMS: The purpose of these group comparisons is to isolate factors that may differentiate those who served in the Gulf who are seeking treatment for health complaints from their treatment seeking counterparts who were not deployed to the Gulf.

METHODOLOGY: The group of patients who were deployed to the Gulf will be compared with those who were not deployed to the Gulf. Data from these groups will also be compared with those from a (non-treatment seeking) research sample of PGW veterans who are being studied using the same neuropsychological instruments. In addition, all of these patients and research subjects will be administered a standardized set of questionnaires and semi-structured interviews that will identify their current health symptoms, pre-existing physical and mental health condition, soci-demographic variables, Gulf experiences, and post PGC-era stressors. These instruments also permit PTSD, MCS, CFS, and other psychiatric disorders to be diagnosed.

EXPECTED PRODUCTS (MILESTONES): To isolate factors that may differentiate those who served in the Gulf who are seeking treatment for health complaints from their treatment-seeking counterparts who were not deployed to the Gulf. This is a step toward understanding the Gulf War Syndrome.

STATUS/RESULTS TO DATE: To date, over 150 study subjects have participated in the study; 160 additional treatment-seekers have also participated. Neuropsychology test data has been entered for each subject following each evaluation. Data entry of psychometrics and questionnaires is ongoing. We expect to complete data collection in the winter of 1998.

PUBLICATIONS: none to

Title: Effects of Pyridostigmine in Flinders Line Rats Differing in Cholinergic Sensitivity

Project #: DoD-33

Agency: DoD

Study Location: Univ. NC, Chapel Hill

Project Status: Ongoing

Research Type: Mechanistic

P.I.: David H. Overstreet, Ph.D.

Research Focus: Pyridostigmine Bromide, Prevention

Start Date (CY): 1996

Est. Completion (CY): 1999

OVERALL PROJECT OBJECTIVE: Test the hypothesis that a genetically based cholinergic supersensitivity might underlie the increased sensitivity of vulnerable human populations, similar to the condition labeled Multiple Chemical Sensitivity (MCS).

SPECIFIC AIMS: Determine strain-dependent effects of acute and chronically administered pyridostigmine. Determine whether chronic pyridostigmine will protect against effects of other anticholinesterase agents. This will be accomplished by developing an animal model with cholinergic supersensitivity which is also more sensitive to a variety of drugs and other chemical agents.

METHODOLOGY: Rats will be treated with pyridostigmine acutely or chronically and growth hormone, body temperature, and locomotor activity will be assessed. The Flinders Line rats developed at Flinders University in Australia by selective breeding for differential responses to the anticholinesterase diisopropyl fluorophosphate (DFP). These rats have been shown to be more

sensitive to directly acting muscarinic agonist, as well as a variety of other drugs including alcohol, diazepam, and nicotine. The heightened sensitivity of this special line of rats to a variety of drugs suggests that they will also be more sensitive to the effects of pyridostigmine, an anticholinesterase drug which was given to soldiers during ODS.

EXPECTED PRODUCTS/MILESTONES: Ultimately, this may lead to the development of challenge test to predict cholinergic sensitivity in soldiers.

STATUS/RESULTS TO DATE: We have confirmed that the FSI, rats exhibit greater increases in serum growth hormone following acutely administered pyridostigmine. In this regard, they resemble depressed individuals, who exhibit a greater growth hormone response to pyridostigmine compared to normal controls. However, this greater sensitivity to pyridostigmine did not translate to altered protection against other anticholinesterases following chronic treatment with pyridostigmine. Rats that were chronically treated with pyridostigmine appeared to exhibit greater short-term decreases in temperature following the acute administration of diisopropyl fluorophosphoric or chlorpyrifos. Thus, chronic treatment with pyridostigmine made the rats more sensitive to the subsequent injection of the anticholinesterases in the periphery, while the other agents inhibit cholinesterases in the brain as well as the periphery. Work this year with centrally acting physostigmine may confirm this.

PUBLICATIONS: Overstreet DH, Daws LC, Schiller GC, Orbach J, Janowsky DS. Cholinergic/serotonergic interactions in hypothermia: Implications for rat models of depression. *Pharmacol Biochem Behav*, 59:59:777-785, 1998.

Overstreet DH, Mason GA, Yang Y, Rezvani AH. Supersensitive growth hormone responses following pyridostigmine challenge in a genetic animal model of depression. Presented at 28th Annual Meeting of the Society for Neuroscience, Los Angeles, CA, Nov. 7-12, 1998, Abstract 189,10.

Overstreet DH, Yang Y, Rezvani AH. Failure of Pyridostigmine Pretreatment to Protect against the Effects of Chlorpyrifos. Presented at Conference on Federally Sponsored Gulf War Veterans' Illnesses Research, Pentagon City, VA June 17-19, 1998.

Overstreet DH, Yang Y, Clark Ejr, Rezvani AH. More rapid decreases in temperature following anticholinesterase challenges in rats pretreated with pyridostigmine. Presented at 16th International Neurotoxicology Conference, Little Rock, AR, September 13-16, 1998.

Overstreet DH, Rezvani AH, Yang Y, Hamed H, Janowsky DS. Animal model of chemical sensitivity involving cholinergic agents. Presented at Toxicology in Risk Assessment Symposium held in Bethesda, MD, 1997.

Overstreet DH, Yang Y, Hamed M, Janowsky DS, Rezvani AH. Strain-and Gender-dependent effects of oxotremorine and pyridostigmine. Presented at Society for Neuroscience, New Orleans, 1997.

Title: Characterization of Emissions from Heaters Burning Leaded Diesel Fuel in Unvented Tents

Project #: DoD-34

Agency: DoD

Study Location: USDE, Albuquerque, NM

Project Status: Ongoing

Research Type: Development

P.I.: Hsu-Chi Yeh, Ph.D.

Research Focus: Environmental Toxicology, Interactions

Start Date (CY): 1996

Est. Completion (CY): 1999

OVERALL PROJECT OBJECTIVE: Estimate the exposure to in-tent pollutants during the Gulf War deployment by simulation of human exposure to aerosols in unvented heated portable kerosene heaters.

SPECIFIC AIMS: Perform physical and chemical characterization of aerosols produced by

kerosene heaters that burns kerosene and jet fuels in an unvented tent, and estimate exposure to lead, combustion products including particulate and gases.

METHODOLOGY: A used Army GP-medium tent was set up for the study. Three types of both heaters and fuels(kerosene, JA1, and JP8 fuels) were used in this study. Aerosols from heaters in an unvented tent were characterized physically and chemically and exposures to particulate matter and combustion gases (CO, NOx, and SO2) were estimated. The aerodynamic particle size distribution measured by a MOUDI impactor. The air exchange rate, a major factor in determining the concentration inside the tent was determined with a SF6 trace gas method. Concentrations of NOx, CO, SO2, and HC were monitored continuously. Both PM-10 and PM-2.5 personal samplers were used to collect particles. Chemical compositions (trace metals as well as nitrates, sulfates, and carbons) of particulate samples collected on filters were analyzed.

EXPECTED PRODUCTS/MILESTONES: Detailed physico-chemical characterization of emissions from in-tent portable heaters were obtained. The choice of heater, fuel type, and air exchange rate are important factors in understanding the exposure and inhalation dosimetry. We planned to complete the experiments in the first two years of the project and chemical analysis, and dosimetry calculations in the third year of the project.

STATUS/RESULTS TO DATE: We have completed all sampling and collection of emission for three types of heaters and three fuels. We found that the air exchange rate in the tent ranged from 1 to 3.5 per hr, and as air exchange rate increased the pollutant concentration decreased. Particle size analysis indicated single or bimodal size distributions with the major peak between 0.1 and 1 μ m. We found CO and particulate concentrations reached the maximum soon after the combustion started, however, NO and SO2 concentrations rose slowly.

PUBLICATIONS: Zhou Y, Cheng YS. Characterization of emissions from kerosene heaters in an unvented tent. Aerosol Sci Technol (submitted)

Title: Feasibility of Investigating Whether There is a Relationship Between Birth Defects and Service in the Gulf War.

Project #: DoD-35

Agency: DoD

Study Location: March of Dimes, Sacramento, CA

Project Status: Ongoing

Research Type: Epidemiology Research

P.I.: John A. Harris, M.D.

Research Focus: Reproductive Health

Start Date (CY): 1996

Est. Completion (CY): 1999

OVERALL PROJECT OBJECTIVE: Conduct a feasibility study to determine how to systematically Monitor birth defects among active and reserve military personnel.

SPECIFIC AIMS: 1) Determine if DoD hospital record computerized information, the data that has been used for many Gulf War studies, is accurate compared to hospital medical record review and baseline birth defects rates in the civilian population collected by the California Birth Defects Monitoring Program; 2) determine if data on Reserve participants in the Persian Gulf war can be linked to California Vital Statistics files, and therefore the California Birth Defects Monitoring Program's registry data.

METHODOLOGY: To address specific aim #1 above, the DoD will provide its hospital record computerized information on all children born to active duty Gulf War veterans posted or stationed in California between May 1991 and May 1994. The California Birth Defects Monitoring Program (CBDMP) will review medical records of all children identified with a birth defect and a random sample of 450 controls to determine the accuracy and completeness of the DoD hospital record computerized information. To address specific aim #2 above, the DoD will provide a roster of reservists deployed to the Gulf War. The CBDMP will determine the feasibility of linking a

sample of this data set on to the California Vital Statistics file and CBDMP files in order to identify children born to and children with birth defects among this reservist population.

EXPECTED PRODUCTS (MILESTONES): 1) The CBDMP receives and analyzes complete data sets from the DoD; 2) CBDMP visits military hospitals and the military storage facilities in St. Louis to review medical records; 3) data is cleaned, coded, and prepared for analysis; 4) data analysis; 5) report prepared for review by DoD and scientific publication.

STATUS/RESULTS TO DATE: The CBDMP finally secured complete files from the DoD in June 1998 and that data has been analyzed. The CBDMP has already received Military Human Subjects Approval to proceed and will be visiting military hospitals and the medical record storage facility in St. Louis as soon as permission is granted.

PUBLICATIONS: none to date

Title: Fatigue in Persian Gulf Syndrome-Physiologic Mechanisms

Project #: DoD-36

Agency: DoD

Study Location: Univ. TX, SW

Project Status: Ongoing

Research Type: Clinical Research

P.I.: Ronald G. Haller, M.D.

Research Focus: Brain & Nervous System, Symptoms/General Health

Start Date (CY): 1996

Est. Completion (CY): 1999

OVERALL PROJECT OBJECTIVE: Test the hypothesis that mechanisms used by muscle cells to use oxygen and produce energy for work may be impaired in patients suffering abnormal muscle fatigue and the inability to exercise.

SPECIFIC AIMS: On the basis of preliminary results of evaluation of symptoms of abnormal fatigability in Gulf War veterans, the researchers hypothesize that the common complaint of abnormal fatigue and exercise intolerance in these patients is attributable to impaired energy production via oxidative phosphorylation. Under this general hypothesis, the researcher will address four specific questions: (1) Is there an abnormality in muscle oxygen utilization or oxygen transport to muscle during exercise in affected individuals? (2) Is there exaggerated metabolic muscle fatigue in exercise consistent with impaired energy production? (3) Is the metabolic and physiologic response to aerobic physical conditioning impaired in these patients? (4) Is there a specific pattern of impaired activities of mitochondrial oxidative enzymes or respiratory chain complexes to account for impaired oxidative metabolism on attenuated increases in oxidative capacity in response to physical training.

METHODOLOGY: Exercise protocols and non-invasive monitors of oxygen transport and utilization as well as detailed muscle biochemistry will be used to identify specific causes of exercise intolerance in patients. The investigators will employ forearm and cycle exercise to determine maximal work and oxidative capacity and to compare fatigue and metabolic responses to similar workloads among patients and age- and weight-matched sedentary control subjects. The study will compare muscle metabolic and physiologic responses to aerobic training in patients and matched control subjects. The oxidative mechanism in blood will be monitored by measurements of blood levels of diffusible metabolites that reflect oxidative capacity; measurement of intramuscular metabolites using ³¹-phosphorus magnetic resonance spectroscopy; and by using infrared spectroscopy.

EXPECTED PRODUCTS (MILESTONES): This study will evaluate the hypothesis that oxidative limitations detected with non-invasive testing are attributable to impaired function of the mitochondrial respiratory chain as assessed biochemically in biopsied muscle.

STATUS/RESULTS TO DATE: Results in studies of 13 veterans with muscle symptoms compared to 11 asymptomatic veterans reveal no statistically significant differences for force generation, fatigue rates, or metabolic responses to forearm exercise. In cycle exercise there is a trend toward lower capacity in symptomatic versus asymptomatic veterans for peak work

(1.51±0.11 watts/kg versus 1.92±0.18 watts/kg) and oxygen utilization (24.8±6.0 ml/kg/min versus 28.0±6.7 ml/kg/min), but these differences fail to reach statistical significance ($p < .05$).

PUBLICATIONS: none to

Title: Neurobehavioral and Immunological Toxicity of Pyridostigmine, Permethrin, and DEET in Male and Female Rats

Project #: DoD-37

Agency: DoD

Study Location: Univ. FL, Gainesville

Project Status: Ongoing

Research Type: Mechanistic

P.I.: Frans van Haaren, Ph.D.

Research Focus: Interactions, Pyridostigmine Bromide, Brain & Nervous System

Start Date (CY): 1996

Est. Completion (CY): 1999

OVERALL PROJECT OBJECTIVE: Determine if neurobehavioral toxicity and immune response alterations are produced by small doses of medical pretreatments and insecticides, alone or in combination, in male and female rats.

SPECIFIC AIMS: This study will test the hypothesis that administration of pyridostigmine bromide (PB), permethrin (PERM) and DEET as single agents, or in combination, results in neurobehavioral toxicity and an altered immune response. It will also test the hypothesis that females are significantly more sensitive to the effects of these agents than are males.

METHODOLOGY: Chemical analyses will determine (1) the metabolism and elimination of PB, permethrin and DEET, alone and in combination, for male and female subjects who participated in the neurobehavioral experiments, and (2) how PB, permethrin and DEET, alone and in combination, affect cholinesterase activity in these same subjects. This information will allow the correlation of plasma concentrations of PB, permethrin and DEET with the behavioral observations. Experiments have been designed to measure various aspects of CNS functioning in the presence of sub-toxic doses of PB, PERM, and DEET in adult male and female rats. Doses for PB will be 3/10/30 mg/kg, for PERM will be 15/30/60 mg/kg and for DEET will be 50/200/500 mg/kg. In addition, plasma concentrations of PB, PERM, and DEET will be determined and their effects on cholinesterase activity will be correlated with CNS functioning. The neurobehavioral analyses will be complemented by an assessment of the immune response in the same subjects, as well as in lymphocytes from health human volunteers. The immunological experiments will be conducted to determine the role of PB, permethrin and DEET and (1) Suppression of the first and second signal transduction pathways in T-lymphocytes, (2) Cytokine expression, and (3) B-lymphocyte function. The neurobehavioral test battery is designed to measure critical aspects of CNS functioning related to symptoms of generalized fatigue, gastro-intestinal disturbance, muscle and joint pain, headaches, and memory loss. The neurobehavioral test battery includes an assessment of spontaneous locomotor behavior and an analysis of overall CNS functioning, motivation and memory performance.

EXPECTED PRODUCTS (MILESTONES): This study extends observations from previous experiments in male rats exposed to toxic doses of pyridostigmine or permethrin to female rats and to the effects of PB, permethrin and/or DEET in combination. This study will also provide additional information on the motivational and memory aspects of CNS functioning.

STATUS/RESULTS TO DATE: see publications.

PUBLICATIONS: Hoy JB, Cody BA, Karlis JL, Schmidt CJ, Tebbett IR, Toffollo S, vanHaaren F, Wielbo D. Pyridostigmine bromide alters locomotion and thigmotaxis of rats: gender effects. Pharmacology Biochemistry and Behavior, 1998 (in review).
vanHaaren F, de Jongh R, Hoy JB, Karlis JL, Schmidt CR, Tebbett Ir, Wielbo D. The effects of acute and repeated pyridostigmine bromide administration on response acquisition with

immediate and delayed reinforcement. Pharmacology Biochemistry and Behavior, 1998 (in press).

Hoy JB, VanHaaren F, Tebbett Ir, Karlix JL. Pyridostigmine bromide affects open field speed and center time in male and female rats. (abstract) Twenty-seventh Annual Meeting of the Society for Neuroscience, New Orleans, LA.

vanHaaren F, Joy JB, Karlix JL, Tebbett Ir. Gulf War illness: effects of pyridostigmine bromide and permethrin alone, or in combination, on response acquisition in male Sprague-Dawley rats. (abstract) Annual Meeting of the Behavioral Toxicology Society, West Palm Beach, FL, 1997.

Karlix JL, Freiburger B, Hoy J, Tebbett IR, Wielbo D, vanHaaren F, Schmidt C. The immunomodulatory effects of chemicals used during the Gulf War. (abstract) Conf. Federally Sponsored GWVs' Illnesses Research, Washington, DC, 1998.

Title: Diagnostic Antigens of Leishmania tropica

Project #: DoD-38

Agency: DoD

Study Location: Inf. Dis. Res. Instit., Seattle, WA

Project Status: Complete

Research Type: Development

P.I.: Steven G. Reed, Ph.D.

Research Focus: Leishmaniasis, Diagnosis

Start Date (CY): 1996

Est. Completion (CY): 1998

OVERALL PROJECT OBJECTIVE: Develop a sensitive method to detect infection with Leishmania tropica, or related species, in military personnel.

SPECIFIC AIMS: There are currently no defined antigens for use in the diagnosis of leishmaniasis. There is an urgent need for sensitive methods to detect infection with Leishmania tropica, or related species, in military personnel. The researchers have developed such tests for other Leishmania species, and have isolated and characterized L. tropica antigens reactive with sera from L. tropica infected Gulf War veterans. This study will attempt to develop similar tests for L. tropica using these recombinant L. tropica antigens. Effort will also be directed at cloning additional L. tropica antigens reactive with L. tropica infection sera and antigens that stimulate T-cells from individuals with L. tropica infection.

METHODOLOGY: Emphasis will be given to expressed genes which are highly sensitive. Inserts will be sequenced for determination of epitopes which may be produced synthetically. Recombinant or synthetic peptides will be evaluated by ELISA for their ability to detect antibody in patient sera. In related studies, cloned gene products will be evaluated for their ability to elicit patient T-cell response in vitro. These evaluations will include proliferation and cytokine production. Emphasis will be placed on finding antigens which are shared between Leishmania species having the ability to elicit strong T-cell responses. Such antigens will be candidates for skin test antigens and vaccine production.

STATUS/RESULTS TO DATE: Several new peptides of Lt-1 antigen have been made and evaluated them as serological reagents. Also, screening of L. tropica genomic libraries for new antigens have been performed, and other candidate antigens have been identified. Evaluation of these antigens on documented viscerotropic L. tropica patients would further this development.

PUBLICATIONS: none to date

Title: A Controlled Epidemiological and Clinical Study into the Effect of Gulf War Service on Servicemen and Women of the United Kingdom Armed Forces

Project #: DoD-39

Agency: DoD

Study Location: King's Coll., UK

Project Status: Ongoing

Research Type: Epidemiology Research

P.I.: Simon Wessely, M.D.

Research Focus: Symptoms/General Health

Start Date (CY): 1996

Est. Completion (CY): 1999

OVERALL PROJECT OBJECTIVE: This research assesses the prevalence of unexplained illnesses, including chronic fatigue-like symptoms, in members of the United Kingdom Armed Forces who were deployed to the Persian Gulf during the Gulf War and who have served, and are serving in Bosnia.

SPECIFIC AIMS: This study is a two stage cohort study. Stage 1 consists of a postal health screening questionnaire to be sent to three groups who were/are in the Armed Forces; those who served in the Persian Gulf, those who served in Bosnia, and those who served in neither the Persian Gulf or Bosnia. The second stage involves performing neuropsychological tests on cases identified by stage 1 and a control group.

METHODOLOGY: This epidemiological study of the prevalence of unexplained illnesses in the populations at risk will use a two-stage design. Stage 1 will be a questionnaire survey of 4,520 Gulf War veterans selected at random, an equivalent sample of Bosnia veterans, and appropriate control groups for each. Stage 2 will involve interview, examination, and testing of all those (approximately 10%) in Stage 1 who fall above a cutoff defining subjective health. Information gathered at Stage 2 will be used to estimate the prevalence of diagnosed and unexplained morbidity, including chronic fatigue symptoms, in UK service personnel, and to calculate whether there is an excess associated with Gulf War and/or Bosnia service. If there is, then the researchers will be able to examine pre-morbid and psychosocial factors which may be implicated in such an increase, as well as identify avenues for further biological and psychosocial research.

EXPECTED PRODUCTS (MILESTONES): Reports and Publications. This epidemiological study should ascertain if service in the Gulf War by UK armed forces personnel was associated with an increase in physical and/or psychological morbidity, and if so, if there is evidence of an increase in either new or ill-defined conditions such as chronic fatigue syndrome, or an illness peculiar to Gulf War service.

STATUS/RESULTS TO DATE: The mailing of the 12,750 questionnaires to serving and discharged personnel has been successfully completed after utilizing intensive tracing methods for the non-respondents. The second phase is currently underway, where those individuals identified by their responses to the questionnaire are invited to the research unit for more details, tests, and examinations.

PUBLICATIONS: Unwin CE, Blatchley N, Coker W, Ferry S, Hotopf M, Hull L, Ismail K, Palmer I, David A, Wessely S. The health of United Kingdom Servicemen who served in the Persian Gulf War. *Lancet*, 353:169-178, 1999.

Ismail K, Everitt B, Blatchley N, Hull L, Unwin CE, David A, Wessely S. Is there a Gulf War Syndrome? *Lancet*, 353:129-182, 1999.

Ismail K, Unwin CE, Hull L, David A, Wessely S. Defining Ill health in three British Military Cohorts. Sponsored by Gulf War Veterans' Illnesses Research, Pentagon City, USA June 17-19, 1998.

Title: Psychological and Neurobiological Consequences of the Gulf War Experience

Project #: DoD-40

Agency: DoD

Study Location: VAMC West Haven. CT

Project Status: Ongoing

Research Type: Clinical Research

P.I.: Steven Southwick, M.D.

Research Focus: Brain & Nervous System, Symptoms/General Health

Start Date (CY): 1998

Est. Completion (CY): 2001

OVERALL PROJECT OBJECTIVE: To determine the nature of memory for traumatic events as they relate to posttraumatic stress disorder (PTSD) symptoms.

SPECIFIC AIMS: To examine the course of memory for traumatic events over time and the brain areas believed to be altered due to trauma.

METHODOLOGY: Longitudinal follow-up of a cohort of veterans of Operation Desert Storm (e.g. questionnaires, ratings scales). MRI study of 20 Desert Storm veterans with PTSD, 20 deployed Desert Storm veterans without PTSD, and 20 non-deployed veterans. This research will continue to follow the course of symptoms of Post-Traumatic Stress Disorder in a population of Gulf War veterans. This study of how such symptoms, as well as memory function, change over time in the veterans will lead to a better understanding of PTSD and the elements of risk that would cause and continue PTSD symptoms over time. The study is in two parts. The first part is a longitudinal descriptive study of trauma-related symptomatology in Gulf War veterans. The second part is an investigation of memory function and hippocampal volume in Gulf War veterans who meet criteria for posttraumatic stress disorder (PTSD). Results of the researchers' previous work with a cohort of Gulf War veterans indicated that there was an overall increase in PTSD symptomatology in the veterans over the first two years following the Gulf War. With regard to memory testing, there were many instances of inconsistent recall for events that were objective and highly traumatic in nature. The data do not support the position that traumatic memories are fixed or indelible and suggest that as PTSD symptomatology increases, so does amplification of memory for traumatic events. This study continues the original Methodology for the fifth, sixth and seventh post-war years. It is anticipated that a high percentage of subjects who have already participated in this research can be recruited to continue in this project and that at least 100 new subjects can also be recruited. It is anticipated that a better understanding of the longitudinal course of trauma-related symptomatology and risk factors for the development and maintenance of these symptoms would have implications for treatment. The second part of the study will examine the possibility that memory deficits in the subject population are relatively broad-based and reflect problems at several levels of information processing, including acquisition, retention and/or retrieval and that these memory deficits may be related to decreased hippocampal volume. Results of this study would make it possible to determine whether these abnormalities are present in a variety of combat populations and whether such abnormalities can be detected at earlier stages than in the previously-studied Vietnam veteran population. Potential relationship to clinical symptomatology and psychosocial functioning will also be investigated.

EXPECTED PRODUCTS/MILESTONES: The investigators expect to show that memory for traumatic events is not consistent over time, and that there may be associated decrease in hippocampal volume in PTSD.

STATUS/RESULTS TO DATE: To date, 12 MRI scans have been completed with veterans with PTSD and 22 MRI scans have been completed with veterans without PTSD. Five participants in the non-deployed veteran group have completed MRI scans. The longitudinal questionnaire is in final revision.

PUBLICATIONS: none to

Title: Evaluation of Muscle Function in Persian Gulf Veterans

Project #: DoD-41

Agency: DoD

Study Location: Univ. PA

Project Status: Ongoing

Research Type: Clinical Research

P.I.: Krista Vandeborne, Ph.D.

Research Focus: Brain & Nervous System. Symptoms/General Health

Start Date (CY): 1996

Est. Completion (CY): 1999

OVERALL PROJECT OBJECTIVE: Investigate the etiology of ongoing chronic fatigue and muscle weakness in Gulf War veterans with unexplained illnesses by evaluating abnormalities in skeletal muscle function.

SPECIFIC AIMS: Evaluate skeletal muscle in Persian Gulf veterans with severe chronic fatigue and Persian Gulf veterans with no medical complaints (healthy controls); determine the relationship between the severity of chronic fatigue in Persian Gulf veterans and the degree of muscle dysfunction; and compare muscle function in Persian Gulf veterans with severe chronic fatigue with muscle function in healthy sedentary civilians and civilians with chronic fatigue.

METHODOLOGY: The goal of this proposal is to evaluate skeletal muscle in Persian Gulf veterans. Our major hypothesis is that Persian Gulf veterans who present a symptom profile consistent with chronic fatigue suffer from muscle dysfunctioning. In addition, we hypothesize that the severity of chronic fatigue in this population is related to the degree of muscle dysfunctioning. To test these hypotheses, a battery of tests are performed on Persian Gulf veterans with severe chronic fatigue (n=50) and on Persian Gulf veterans who were deployed but who have no medical problems (n=50). The evaluation will consist of the following measurements: 1. Functional assessment as reflected in isometric and isokinetic measures; 2. In vivo metabolic measures as determined by ³¹P-Magnetic Resonance spectroscopy (MRS); 3. In vivo morphological measures as determined via Magnetic Resonance Imaging (MRI); 4. Histological analyses of muscle biopsies; 5. Electromyographic evaluation of motor unit recruitment; 6. Muscle enzyme levels; 7. Genetic screening.

EXPECTED PROJECTS (MILESTONES): This project runs over three years. The first 6 months served to recruit the subjects and to train the necessary personnel. The following two years are being used to collect all the data at a rate of approximately 1 subject/week (at the end of the first year 13 Persian Gulf veterans with chronic fatigue and 13 healthy veterans will have been tested). At the end of the second year, 24 more subjects with chronic fatigue and 24 healthy controls will have been tested. For the first 6 months of the third year the final 13 veterans with chronic fatigue and 13 controls will be tested. Data collection will be completed at the end of 2.5 years. The final 6 months of this 3 year period will be used to analyze and interpret the data.

STATUS/RESULTS TO DATE: In the process of acquiring data.

PUBLICATIONS: Vandenborne K, Walter G, Lenrow D, Leigh JS, Fishman AP. Skeletal muscle in Persian Gulf veterans. J Chronic Fatigue Syndrome, 2(2/3):137-138, 1996.

Title: The Symptomatic Persian Gulf Veterans Protocol: An Analysis of Risk Factors with an Immunologic and Neuropsychiatric Assessment

Project #: DoD-42

Agency: DoD

Study Location: VAMC Birmingham

Project Status: Ongoing

Research Type: Clinical Research

P.I.: Warren Blackburn, Jr. M.D.

Research Focus: Symptoms/General Health, Immune Function, Brain & Nervous System

Start Date (CY): 1996

Est. Completion (CY): 2000

OVERALL PROJECT OBJECTIVE: Determine if there are fundamental neuropsychiatric or immunologic abnormalities in Gulf War veterans which might be associated in a case control study with their service in the Gulf.

SPECIFIC AIMS: The specific goals of this proposal are to: 1) evaluate self-reported conditions in Persian Gulf veterans to determine if they represent described and accepted medical conditions and/or are associated with objective findings which would validate these symptoms; 2) evaluate

symptomatic Gulf war veterans to determine risk factors which may identify veterans who have reported symptoms; and, 3) evaluate in a controlled fashion symptomatic, but undiagnosed, Persian Gulf war veterans to determine if there is a definable immunologic or neuro-psychiatric abnormality.

METHODOLOGY: This study will determine whether specific immunological or neuropsychiatric abnormalities can be detected in GW participants who are symptomatic, but in whom no specific diagnosis has as yet been made. These studies may uncover an immunological basis for some of the broad types of symptoms reported by GW veterans. This proposal attempts to get at the heart of the problem cited in the Institute of Medicine (IOM) report, that GW veterans' mystery illnesses may not be a single illness and that it is important to study various possible causes and the interactions between causes. The Birmingham VAMC has been very active in evaluating patients returning from the Persian Gulf war. Approximately 1400 individuals have been seen and have had evaluations. Of these, about 10-15% are asymptomatic. Of the remaining 85%, approximately 90% have a diagnosable illness. A number of Persian Gulf veterans, which are conservatively estimated as 100 of those seen at the BVAMC, are symptomatic and do not have defined illnesses. The use of advanced Methodology in the imaging, immune, mycoplasma and oxidant injury study is important. Sample sizes are adequate and support for statistical analysis is an integral part of the proposal.

EXPECTED PRODUCTS (MILESTONES): This study should answer the questions regarding whether veterans from the Persian Gulf who have undefined illnesses have evidence of exposure to Mycoplasma more often than the control groups and if there are differences in laboratory measures of cellular immune responsiveness.

STATUS/RESULTS TO DATE: 1,541 Persian Gulf War veterans have been enrolled in this study who were also enrolled in the Birmingham registry. Charts have been available for 1,347 and they have been carefully reviewed and have had second physician review. One hundred and seventy-seven have illnesses involving more than two organ systems that have not been accounted for with a defined illness.

Second, we have received and have categorized the list of veterans who served in the Persian Gulf from the Birmingham catchment area. This is the group of individuals who will represent our in-theater, asymptomatic individuals and will be drawn from a list of 1,383 in theater Alabama veterans.

Third, we identified individuals in the Birmingham catchment area who were not in theater, but who have applied for disability. These individuals represent the second control group we have identified as a listing of 314 veterans.

From these three groups, sixty-seven have been contacted so far and 65 have agreed to participate in the study. We have evaluated approximately 20 of these and expect to recruit the remainder during the next approximately 12-18 months.

PUBLICATIONS: none to

Title: Investigation of Seminal Plasma Hypersensitivity Reactions

Project #: DoD-44

Agency: DoD

Study Location: Univ. Cincinnati MSC

Project Status: Ongoing

Research Type: Clinical Research

P.I.: Jonathan Bernstein, M.D.

Research Focus: Reproductive Health, Symptoms/General Health, Immune Function

Start Date (CY): 1996

Est. Completion (CY): 1999

OVERALL PROJECT OBJECTIVE: The purpose of this project is to investigate Persian Gulf War (GW) veterans and their sexual partner(s) who are experiencing a burning sensation after contact with semen since returning from the Persian Gulf.

SPECIFIC AIMS: Military and veteran sources will be surveyed to obtain a population of women

who developed vaginal burning syndrome after their sexual partners returned from the Persian Gulf.

METHODOLOGY: The initial phase of this project has concentrated on identifying the population and characterizing the nature of their symptoms using screening questionnaires. Questionnaires assessing for post-traumatic stress disorder were also included. Subjects were identified through local and regional veterans hospital Gulf War screening physicians, in response to media press releases pertaining to this investigation and through a "Burning Semen Syndrome" (BSS) web page on the internet. A pilot project was conducted to more thoroughly investigate a small group of PGW veterans in local proximity to the Cincinnati Veterans Administration Hospital (VAH). This evaluation included more detailed questionnaires related to their medical history before and after their tour of duty in the Persian Gulf, potential exposures while there and the nature of their burning semen symptoms. Screening blood tests were performed on both the PGW veteran and their sexual partner to exclude underlying etiologies such as sexually transmitted diseases. Semen was obtained from each male participant and cultured to exclude viral, bacterial and fungal infections. Each female underwent a pap smear with cervical and vaginal cultures to exclude viral, bacterial and fungal infections. Both subjects were skin tested to perennial and seasonal allergens to assess their atopic state. Each subject was also skin tested to the male's whole semen to diagnose or exclude an immediate type allergic response. Serum from each subject was also used to determine if IgG, IgE and IgA specific antibodies were being produced in response to seminal plasma proteins using an ELISA method. Based on the preliminary results from the vaginal cultures, a sensitive and specific Polymerase Chain Reaction (PCR) assay was developed to detect the presence of *Ureaplasma urealyticum* in the veterans' semen.

EXPECTED PRODUCTS (MILESTONES): If the hypotheses are correct, 1) women with seminal plasma hypersensitivity and vaginal burning syndrome will demonstrate immediate cutaneous reactivity to seminal plasma protein fractions; 2) one or more seminal plasma proteins that cause localized vaginal symptoms in our civilian group of women will also cause similar symptoms in women with Gulf War vaginal burning syndrome; and 3) the ability of seminal plasma proteins to regulate (either stimulate or inhibit) the humoral, cellular or secretory immune responses after exposure to physical and chemical factors encountered in the Gulf War.

STATUS/RESULTS TO DATE: Preliminary findings: A total of 134 individuals responded to the screening questionnaires. All of the respondents were male; 87% of their female sexual partners experienced a burning sensation after contact with semen while only 38% of the males experienced burning after contact with their own semen. There did not appear to be any correlation between BSS and post-traumatic stress disorder among those that completed all of the questionnaires. Six male PGW veterans and five of their respective sexual partners participated in the pilot study (one of the PGW veteran's sexual partner declined participation as she was asymptomatic). Four of the six male PGW veterans and two of the five female sexual partners exhibited positive skin tests consistent with atopy. None of the participants or their sexual partners elicited positive skin test reactions to their whole semen, however, for 5 of the 6 males tested, specific serum IgG or IgE antibodies to seminal plasma proteins were detected using an ELISA method. IgG or IgE antibodies to seminal plasma proteins were also detected in 5 of the 6 women tested. Three women had *ureaplasma urealyticum* isolated from their cervical cultures, one grew streptococcus Group B from their cervical culture and one had an active yeast infection. Two of the women had significant ANA titers and one had an increased sedimentation rate. Of the five men evaluated in the pilot study, one tested positive for *ureaplasma urealyticum* by utilizing PCR. Investigation is also centered on identifying whether bacteriostatic proteins and other bacteriostatic compounds (I.e. Zinc sulfate) are deficient in their semen making their sexual partner more susceptible to infection. An extensive evaluation of additional PGW veterans and sexual partners has expanded beyond the pilot group. Two additional men have tested positive for *ureaplasma urealyticum*. Also one additional male and 3 females have had specific serum IgG or IgE antibodies to seminal plasma proteins were detected using an ELISA method.

Active recruitment will continue during the next 10 months to identify a larger number of PGW couples with BSS who will undergo the more extensive medical evaluation. Individual hazardous exposure data for participants is being obtained and considered in the final analysis. Cohort control groups consisting of asymptomatic PGW veterans deployed to the Persian Gulf and their

sexual partners as well as nonveteran couples diagnosed with seminal plasma hypersensitivity are also being recruited to participate in this project. In addition, a separate epidemiological study has been initiated to determine the incidence rate of seminal plasma hypersensitivity in the general population. As a co-investigator in the VA cooperative study 458 the opportunity is present to obtain the incidence rate of burning semen symptoms in 22,000 individuals in PGW families. Initial findings suggest that while infection may be playing a role in causing burning semen symptoms, an immunologic etiology appears likely in the many of the individuals evaluated thus far and will be pursued further. In addition, initial findings of other VA researchers suggest that the role of Depleted Uranium and pyridostigmine bromide must also be investigated. All subjects with evidence of infection will be offered treatment with appropriate antibiotics and/or antifungals to determine if the burning symptoms are attenuated. Women demonstrating antibody reactions to their partners semen will be treated by rapid desensitization. More detailed evaluation of a larger population of PGW veterans with BSS and cohort control groups is necessary before linkage of BSS to service in the Persian Gulf can be established.

PUBLICATIONS: Bernstein JA, Martin RLM, Lummus ZL. Localized Human Seminal Plasma Hypersensitivity: A Potential Model for Gulf War 'Burning Semen Syndrome.' *Fundamental and Applied Toxicology*, 36:201, 1997

Title: Physical and Emotional Health of Gulf War Veterans Women

Project #: DoD-45

Agency: DoD

Study Location: Univ. MI, Ann Arbor

Project Status: Ongoing

Research Type: Clinical Research

P.I.: Penny Pierce, Ph.D., R.N.

Research Focus: Symptoms/General Health, Brain & Nervous System, Reproductive Health

Start Date (CY): 1996

Est. Completion (CY): 1999

OVERALL PROJECT OBJECTIVE: This study will compare the prevalence of general and gender specific health problems in 900 Air force women deployed to the Gulf and 900 Air Force women deployed elsewhere.

SPECIFIC AIMS: To assess whether the prevalence of health problems remain elevated in women deployed to the Gulf at six years post deployment, after controlling for potentially confounding factors such as age, reproductive history, sexual history, and lifestyle factors.

METHODOLOGY: This research is to describe the incidence, prevalence, and nature of symptoms associated with Persian Gulf War service within a randomized sample of Air Force women. Our design is developed on previous experience with two studies of Gulf War veteran women as well as the recommendations of numerous military and civilian scientific groups established to address this body of research. Specifically, in previous studies we have used a comparison group of women deployed to sites other than the theater of the war under the assumption they were physically fit for duty and would provide a better comparison than those who did not deploy perhaps for physical health reasons. Further sampling strategies will follow the recommendations for both the National Institutes of Health (NIH, 1994) and the Institute of Medicine (IOM, 1995; Roberts, 1995), who both call for well-designed epidemiologic studies. In both our previous studies we used a randomized stratified factorial design (theater vs. non-theater; parent vs. non-parent; and component (active, guard, and reserve). Similar sampling procedures will be used in this study in order to control for potentially confounding effects of component and paternal status. This study will also include detailed information on sexual, reproductive, and menstrual history to allow for more complete analyses of findings. Sample size and power will detect differences observed in previous studies. Data collection involves recruiting the study participants and maximizing the response rate while minimizing attrition. A single

comprehensive questionnaire will provide basic demographic, socioeconomic, and background information and include measures of general physical health and measures of gender-specific health. Mental health measures include assessment of depression, somatization, and 8 PTSD specific to Gulf War service and will include a stressful life event measure. Data processing includes all appropriate range and logic checks. Statistical analyses include descriptive statistics, cross tabulations with chi-square and t-tests, distributions, and multiple regression analyses for health outcomes and deployment status.

EXPECTED PRODUCTS (MILESTONES): Three project years: 1) all planning and pre-testing during year 1; one-half of data collection (mail administration) in year 1 and the second half in year 2; all coding and data entry in year 2; and the final report and electronic data file as deliverables.

STATUS/RESULTS TO DATE: Data collection is in progress.

PUBLICATIONS: none to date

Title: Exploratory Data Analysis with the CCEP Database

Project #: DoD-46

Agency: DoD

Study Location: Naval Post-Graduate School, Monterey, CA

Project Status: Complete

Research Type: Epidemiology Research

P.I.: Hemant Bhargava, Ph.D.

Research Focus: Symptoms/General Health

Start Date (CY): 1995

Est. Completion (CY): 1997

OVERALL PROJECT OBJECTIVE: To systematically examine the database for interesting relationships, and to find, with a high level of confidence, the most interesting ones without having to do an exhaustive search.

SPECIFIC AIMS: 1) to develop a generic computer package to do exploratory data analysis using genetic algorithms to decide which attributes to consider in the analysis; 2) to apply this package on a number of specific application runs relevant to the Gulf War Syndrome problem; 3) to produce, analyze, and comprehensively document the results of these runs, and produce a set of findings and conclusions.

METHODOLOGY: For the search problem over the space of attributes found in the CCEP database, the main search Methodology is based on genetic algorithms. The overall solution Methodology consists of exploratory data analysis and knowledge discovery in databases.

EXPECTED PRODUCTS (MILESTONES): 1. The computer software: a) a hypothesis generator module; b) a filtering module; and c) a results presentation and interpretation module.

STATUS/RESULTS TO DATE: Version 1.0 of the Hypothesis Generator Module is complete, tested, and applied in a series of production runs.

PUBLICATIONS: none to date

Title: Study of Mycoplasmal Infections in Gulf War Veterans

Project #: DoD-47

Agency: DoD

Study Location: WRAIR, Wash. DC

Project Status: Complete

Research Type: Clinical Research

P.I.: Raymond Chung, MD, COL

Research Focus: Diagnosis

Start Date (CY): 1995

Est. Completion (CY): 1998

OVERALL PROJECT OBJECTIVE: This study was designed to find evidence for mycoplasmal Infections in symptomatic Operation Desert Storm veterans, by comparing the rate of seroconversion in symptomatic veterans as compared to healthy Gulf veterans and by culturing for Mycoplasma organisms in symptomatic and healthy Gulf veterans.

SPECIFIC AIMS: See objectives.

METHODOLOGY: This study has two parts: (1) Sera from 200 symptomatic Gulf veterans and 200 healthy Gulf veterans, stored in the U.S. Armed Forces HIV Repository are used. Sera prior to and after deployment to South West Asia are tested for antibody to M. fermentans, M. genitalium, and M. penetrans. (2) Blood, urine, oropharyngeal, and rectal samples in 100 consecutive veterans undergoing evaluation at WRAMC and 100 asymptomatic veterans are tested for evidence of mycoplasmal infection by culture, serology and PCR.

EXPECTED PRODUCTS (MILESTONES): August 1996 - Initial Part One results; March 1997 – Addendum Part One results; August 1997 - Part Two complete.

STATUS/RESULTS TO DATE: Part one of the study was done using 151 cases and 151 age and gender matched controls. There was no statistically significant difference in the seroconversion rate to any of the aforementioned Mycoplasma. However the seroconversion rate was unexpectedly low: 1.5% for controls and 3.0% in cases for M. fermentans. The odds ratio (OR) for cases compared to controls was 2.0 with a confidence interval of 0.4 - 10.9. In order to be able to detect an OR of 2 with a background seroconversion rate of 1 - 2% with 80% power, a larger study needs to be done. An addendum to the protocol has been submitted. The size of part one of the study was increased to 718 symptomatic cases and 2233 healthy Gulf veterans under the addendum. 34 out of 718 cases (4.8%) and 116 out of 2233 (5.2%) were positive for M. fermentans specific antibody. There was no difference in the seroconversion rate (1.1% for cases, 1.2% for controls). Part two of the study was halted because of slow accrual. 73 symptomatic veterans and 76 controls entered into the study. Results indicate a very low rate of isolation of M. fermentans. Manuscripts describing both parts of the study are prepared and are in review by the authors and will be submitted for publication shortly.

PUBLICATIONS: none to date

Title: Assessment of Genomic Instability via Chromosome 7 Inversion Frequency in a Gulf-War Syndrome Cohort vs Selected Control Groups

Project #: DoD-48

Agency: DoD

Study Location: WRAIR, Wash. DC

Project Status: Complete

Research Type: Clinical Research

P.I.: William Fishbein, M.D., Ph.D.

Research Focus: Immune Function

Start Date (CY): 1995

Est. Completion (CY): 1997

OVERALL PROJECT OBJECTIVE: This study is designed to determine the frequency of chromosome 7 inversions in blood lymphocytes of four groups of volunteers: 1) symptomatic Gulf War veterans with findings consistent with a diagnosis of autoimmune or collagen vascular disease, 2) Gulf War veterans without abnormalities, 3) symptomatic volunteers with autoimmune or collagen vascular disease, and 4) healthy volunteers with no Gulf War exposure.

SPECIFIC AIMS: See objectives.

METHODOLOGY: Blood was obtained from twenty five consecutive cases of each of the four groups. The samples were examined for the chromosome 7 inversion blindly in the laboratory of Dr. William Fishbein, an investigator from the AFIP. After the 100 samples the investigators were unblinded and a statistical analysis will be done.

EXPECTED PRODUCTS (MILESTONES): March 1997 - collection complete; April 1997 - Analysis complete.

STATUS/RESULTS TO DATE: This study of 102 cases has now been completed. after data

distribution, breaking of the code, and statistical analysis. There was adequate matching of age, sex, and race, and no evidence was found for genetic instability among symptomatic Gulf War veterans, or any of the other groups tested; a preliminary report was presented at the Experimental Biology meeting in San Francisco in April, 1998.

PUBLICATIONS: none to date

Title: Diagnosis and Dosimetry of Exposure to Sulfur Mustard: Development of Standard Operating Procedures and Exploratory Research on Protein Adducts

Project #: DoD-49

Agency: DoD

Study Location: TNO Prins Maruits Lab, Rijswijk, Netherlands

Project Status: Ongoing

Research Type: Development

P.I.: Hendrick Benschop, Ph.D.

Research Focus: Chemical Weapons, Diagnosis

Start Date (CY): 1996

Est. Completion (CY): 2000

OVERALL PROJECT OBJECTIVE: Develop procedures to assess mustard vesicant agent levels (which are highly reactive and show little persistence in vivo) in patients, based on estimation of exposure levels from levels of sulfur mustard adducts to DNA and proteins in tissue samples. The principal objective of this research is to develop methodologies that can be readily adapted for use in the field.

SPECIFIC AIMS: Three quantitative assessment procedures will be developed and tested in one set of animal experiments for mutual assay validation. There are: 1) immunochemical assays of sulfur mustard adducts to DNA in human blood and skin; 2) GC/MS determination of the N7-adduct of sulfur mustard with guanine (N7-HETE-Gua) in human blood, skin, and urine; and, 3) GC/MS determination of the sulfur mustard adduct to the N-terminal valine in blood hemoglobin. The practical applicability of the assays will be confirmed by demonstrating the assays at an independent institute.

METHODOLOGY: The feasibility of this project was proved in the previous research where it was proposed to use ³⁵S-sulfur mustard to isolate the adducts. This technique was fruitful with protein adducts in research with hemoglobin. It has been effective in labeling loss of heterozygosity. There is no reason to believe it would not work with albumin and keratin.

EXPECTED PRODUCTS (MILESTONES): 1) Standard operating procedures for immunoslotblot assay and modified Edman procedure for analysis of sulfur mustard adducts to DNA and hemoglobin, respectively. 2) Mass spectrometric and immunochemical methods for detection of sulfur mustard adducts to hemoglobin, albumin and keratin.

STATUS/RESULTS TO DATE: Experiments were carried out to develop standard operating procedures for the immunoslotblot assays of sulfur mustard adducts to DNA in human blood and skin and for a modified Edman degradation for the determination of sulfur mustard adducts to the N-terminal valine in hemoglobin. Both procedures were shortened significantly, and the sensitivity of both procedures was improved. Currently, standard operating procedures are drawn up for both assays.

Further exploratory research was performed for hemoglobin, albumin and keratin in order to develop suitable immunochemical and mass spectrometric methods for detection of sulfur mustard adducts of these proteins. In case of hemoglobin, partial sequences containing an alkylated histidine residue were synthesized and used as haptens. Several monoclonal antibodies have been obtained which recognize in a direct ELISA adducts in hemoglobin treated with 50 mM SULFUR MUSTARD. Upon exposure of human blood to various concentrations of [¹⁴C] sulfur mustard we found that approximately 20% was covalently bound to albumin. One of the alkylated peptides, resulting from tryptic digestion of albumin, i.e., T5 containing an alkylated cysteine, could sensitively be detected with LC-MS-MS analysis (greater or equal to 15 pg absolute). This peptide has been synthesized and is now being used as hapten in order to raise antibodies.

Partial keratin sequences have been synthesized containing an alkylated glutamic or aspartic acid residue and were used as haptens. Several clones are now available producing antibodies which recognize keratin-sulfur mustard adducts. This opens the possibility to develop an immunochemical detection method of sulfur mustard exposure which can be applied directly on the skin.

PUBLICATIONS: Benschop HP, Van der Schans GP, Noort D, Fidder A, Mars-Groeneveld RH, De Jong LPA. Verification of exposure to sulfur mustard in two casualties of the Iran-Iraq conflict. *J Anal Tox*, 21:249-251, 1997.

Noort D, Hulst AG, Trap HC, De Jong LPA, Benschop HP. Synthesis and mass spectrometric identification of the major amino acid adducts formed between sulphur mustard and hemoglobin in human blood. *Arch Toxicol*, 71:171-178, 1997.

Noort D. Mass spectrometric detection of sulfur mustard adducts to DNA and hemoglobin. Abstract book "5th European Meeting on Mass Spectrometry in Occupational and Environmental Health". Nijmegen, The Netherlands, 1997.

Fidder A, Noort D, Benschop HP. A convenient synthesis of [¹⁴C], 1-thiobis (2-chloroethane), [1+ C]sulfur mustard. *J Label Comp Radiopharm*. (in press).

Title: Toxicokinetics of O-Ethyl S-(2-Diisopropylaminoethyl) Methylphosphonothioate [(+)-VX] in Rats, Hairless Guinea Pigs and Marmosets - Identification of Metabolic Pathways

Project #: DoD-50

Agency: DoD

Study Location: TNO Prins Marits Lab, Rijswijk, Netherlands

Project Status: Ongoing **Research Type:** Mechanistic

P.I.: Hendrick Benschop, Ph.D.

Research Focus: Chemical Weapons

Start Date (CY): 1996

Est. Completion (CY): 1999

OVERALL PROJECT OBJECTIVE: Develop Methodology for and clarify the toxicokinetics of VX stereoisomers in laboratory animals.

SPECIFIC AIMS: Develop Methodology for and clarify the toxicokinetics of VX stereoisomers in laboratory animals.

METHODOLOGY: VX enantiomers will be resolved using a detection apparatus to be developed early in the study. The sensitivities of gas chromatographic analysis in combination with mass spectrometry will be compared with that for micro-liquid chromatography employed with a chiral stationary phase. Thermionic and/or flame photometric detection limit of 10 pg per enantiomer. Once selected, the system will be used to identify and quantify +/- VX metabolites generated in vitro in blood, and from liver and skin homogenates from animals, and, if possible, human tissue. In vivo toxicokinetics and metabolism will be determined in the blood of anesthetized, atropinized, and artificially ventilated hairless guinea pigs. The initial time frame for blood sampling will be up to 60 minutes after intoxication and will be modified as appropriate.

EXPECTED PRODUCTS (MILESTONES): Successful completion of this research would enhance the understanding of VX toxicokinetics and metabolic elimination. This information is important because of uncertainties in current pretreatment strategies stemming from differences between VX and its G agent counterparts. Among military chemical agents, VX is the most specific for acetylcholinesterase and is the most toxic. It is also persistent in the field and offers the greatest hazard to military personnel following direct exposure or indirect exposure from the handling of contaminated individuals or materials. It is anticipated that the toxicokinetics of VX will differ from its G agent counterparts.

STATUS/RESULTS TO DATE: Methodology has been developed to analyze biological samples for the (+) and (-) enantiomers of VX at levels > 1 ng VX-enantiomer/ml using chiral liquid chromatography and electrochemical detection. Preliminary toxicokinetic experiments in guinea

pigs at an iv dose of 55 mg/kg, corresponding with 2 LD50, indicate (i) hardly any stereospecificity in the sequestration of the two enantiomers, and (ii) at least one order of magnitude higher blood levels of the agent at 2 hours after administration than in the case of G-agents at comparable dosages, indicating in vivo persistence of VX.

For metabolic studies, 35S-(±)-VX has been synthesized, as well as potential metabolites. Metabolic studies of the isotope-labeled VX in liver homogenates of guinea pigs show a 20% decrease of VX after 12 hours of incubation at 37° C. Identification of metabolites is under way using HPLC with radiometric and mass spectrometric detection. A method has been developed to determine one of the potential metabolites, i.e., O-ethyl methylphosphonic acid (EMPA), utilizing derivatization followed by gas chromatographic analysis.

PUBLICATIONS: none to date

Title: Transgenic Engineering of Cholinesterases: Tools for Exploring Cholinergic Responses

Project #: DoD-51

Agency: DoD

Study Location: Hebrew Univ of Jerusalem, Israel

Project Status: Ongoing

Research Type: Mechanistic

P.I.: Hermona Soreq, Ph.D.

Research Focus: Chemical Weapons, Prevention

Start Date (CY): 1996

Est. Completion (CY): 2000

OVERALL PROJECT OBJECTIVE: To assess the potential of transgenic cholinesterases to protect organisms against anticholinesterase neurotoxicity.

SPECIFIC AIMS: Our previous work has demonstrated that overexpression of a specific alternative variant of the acetylcholine-hydrolyzing enzyme acetylcholinesterase (AChE) in brain protects transgenic mice against the acute toxicity of organophosphate (OP) poisons. However, we also observed that chronic overexpression of AChE in the central nervous system (CNS) may promote delayed impairments in cognitive and neuromotor function. Our objectives, therefore, are to determine the specific cholinesterase isoforms and mode(s) of expression conferring the greatest level of protection against OP poisoning with the least side effects. To this end, we aim to develop animal models for testing the protective value and potential side-effects of transgenic overexpression of various human cholinesterases. In addition, we shall search for genetic factors conferring variability in the susceptibility of individuals to OP intoxication.

METHODOLOGY: To study the protective effects of overexpressed AChE on the nervous system, we established transgenic mice carrying two copies of the human gene encoding the synaptic form of AChE. These mice display elevated levels of AChE in central cholinergic neurons normally expressing this enzyme and reduced sensitivity to cholinergic intoxication. However, despite apparently normal embryonic and early post-natal development, AChE-transgenic mice exhibit delayed-onset, progressive impairments in central cognitive functions such as learning and memory, and in neuromotor function. To determine the protective value of alternative AChE isoforms with different subcellular and tissue-specific patterns of expression, and to dissect the mechanism(s) through which excess AChE mediates late onset neuropathologies, we are establishing additional lines of transgenic mice expressing soluble or inactive forms of the human protein. Massive overexpression was observed in muscle and milk, but very limited excess in brain. However, clear neuropathology could be detected in the somatosensory cortex of mice expressing either inactive or active transgenic synaptic AChE, but much less so with the soluble enzyme. This demonstrates that the long term effects of overexpression are not solely dependent on the catalytic activity of the enzyme, but depend on specific alternative C-terminal peptide domains which exert more active roles in mediating neurodegenerative processes. To investigate possible genetic polymorphisms affecting sensitivity to anticholinesterases we are studying upstream regulatory sequences in the human AChE gene promoter.

EXPECTED PRODUCTS (MILESTONES): This project is designed to select the AChE variant(s)

with the greatest potential to protect an organism from the acute toxicity of OP poisons, while elucidating the mechanisms of delayed neuropathologies induced by chronic overexpression of AChE in the CNS. These studies will therefore allow us to develop strategies for the safe use of transgenic cholinesterases as part of our chemical defense arsenal.

STATUS/RESULTS TO DATE: We have constructed and expressed a variety of catalytically active and inactive variants of AChE in *Xenopus* oocytes and embryos, identified neurite growth-promoting activities of these variants and shown this activity to be independent of acetylcholine hydrolysis. These constructs were then used to establish new lines of transgenic mice which express the transgenes. DNA encoding an antisense RNA targeted against rodent AChE was used to prove functional redundancy in neuritogenesis between AChE and the AChE-homologous protein neuroligin. In normal mice, we found that both acute psychological stress and Ops induce AChE overproduction, conferring delayed protection from AChE inhibitors. Next, the protective activity of the different transgenes to OP poisons will be tested, together with the long-term performance of these mice in tests of cognition and neuromotor function. DNA samples are also being collected from human subjects presenting hypersensitivity to acetylcholinesterases used in medical or agricultural settings and searching for possible allelic variations in either the AChE or BCHE gene which might be correlated with adverse reactions to these compounds. To date, we identified a single point mutation in the human BCHE gene coding sequence and a rare polymorphism in the human AChE gene upstream promoter region that may confer hypersensitivity to a variety of cholinesterase inhibitors

PUBLICATIONS: Grifman M, Gralyam N, Seidman S, Soreq H. Functional redundancy of acetylcholinesterase and neuroligin in mammalian neuritogenesis. *Proc Natl Acad Sci*, 95:13935-13940, 1998.

Kaufer D, Friedman A, Seidman S, Soreq H. Acute stress facilitates long-lasting changes in cholinergic gene expression. *Nature*, 393:373-377, 1998.

Sternfeld M, Ming G, Song H, Sela K, Timberg R, Poo M, Soreq H. Acetylcholinesterase enhances neurite growth and synapse development through alternative contributions of its hydrolytic capacity, core protein, and variable C termini. *J Neurosci*, 18:1240-1249, 1998.

Sternfeld M, Patrick JD, Soreq H. Position effect variegations and brain-specific silencing in transgenic mice overexpressing human acetylcholinesterase variants. *J Physiol (Paris)*, 92:249-256, 1998.

Grisaru D, Lev-Lehman E, Shapira M, Chikin E, Lessing LB, Eldor A, Eckstein F, Soreq H. Human osteogenesis involves differentiation-dependent increases in the morphogenetically active 3 alternative splicing variant of acetylcholinesterase. *Molec Cell Biol*, 1999 (in press).

Kaufer D, Friedman A, Seidman S, Soreq H. Anticholinesterase induce multigenic transcriptional feedback response suppressing cholinergic neurotransmission. *Chem Biol Interact* (in press).

Kaufer D, Friedman A, Soreq H. The vicious circle: long lasting transcriptional modulation of cholinergic neurotransmission following stress and anticholinesterase exposure. *The Neuroscientist* (in press).

Andres C, Seidman S, Beeri R, Timberg R, Soreq H. Transgenic acetylcholinesterase induces enlargement of murine neuromuscular junctions but leaves spinal cord synapses unchanged. *Neurochem Int*, 32:449-456, 1998.

Beeri R, Le Novère Mervis R, Huberman T, Grauer E, Changeux JP, Soreq H. Enhanced hemicholinium binding and attenuated dendrite branching in cognitively impaired acetylcholinesterase-transgenic mice. *J Neurochem*, 69:2441-2451, 1997.

Andres C, Beeri G, Friedman A, Lev-Lehman E, Henis S, Timberg R, Shani M, Soreq H. Acetylcholinesterase-transgenic mice display embryonic modulations in spinal cord choline acetyltransferase and neurexin 1b gene expression followed by late onset neuromotor deterioration. *Proc Natl Acad Sci*, 94:8173-8178, 1997.

Title: Female Gender and Other Potential Predictors of Functional Health Status Among Persian Gulf War Veterans

Project #: DoD-52

Agency: DoD

Study Location: VAMC Boston

Project Status: Ongoing

Research Type: Epidemiology Research

P.I.: Jessica Wolfe, Ph.D.

Research Focus: Brain & Nervous System, Symptoms/General Health

Start Date (CY): 1995

Est. Completion (CY): 1999

OVERALL PROJECT OBJECTIVE: The primary objective of this study is to identify and describe the effects of potential predictors on the functional health status and health perceptions of male and female veterans approximately four years after their deployment to the Persian Gulf; and to examine if and how identified risk factors differ between female and male veterans. A secondary objective of the proposed project is to ascertain the prevalence of multiple chemical sensitivity (MCS)-like symptoms reported among this population, and to explore risk factors for the development of this syndrome.

SPECIFIC AIMS: The more specific goal of this study is to use the obtained data to significantly advance DoD screening and intervention efforts aimed at enhancing positive military and post-deployment adaptation among soldiers, especially women. With women's rapidly increasing representation in the U.S. Armed Forces, there is a pressing need to investigate these relationships, and to understand factors that might be distinctively associated with women's well-being, both during and following war-time deployment.

METHODOLOGY: By using cross-sectional and longitudinal data from an existing, carefully followed military cohort and by expanding predefined measures of health symptoms, and health perceptions, this investigation is conducting analyses that focus directly on: (a) defining the set of reported environmental and psychosocial combat-theater exposures and physical health variables associated with female and male soldiers recent deployment; and, (b) describing the relationship of these variables to functional status and self-reported physical health. A primary emphasis is on investigating the role or impact of gender, specifically, whether female gender is a significant factor in predicting either functional health status or health perceptions. Data analyses will proceed through a series of three major stages. The initial stage involves generation of descriptive analyses for all variables of interest and will permit comparison of responses between women and men. The second stage involves the determination of bivariate odds ratios of gender, PTSD diagnostic status, and environmental exposure on functional health status. The third stage involves multivariate regression procedures to examine the effects of gender, PTSD and reported environmental exposure on functional status and on self-reported health.

In addition, an exploratory analysis will be conducted of the comprehensive symptom review results with respect to the diagnosis of Multiple Chemical Sensitivity. One of the first steps in ascertaining the prevalence of MCS-like symptoms among our study population is developing a survey instrument that can be used to identify subjects with MCS-like symptoms. To date, there is only one survey instrument that has been validated to assess MCS symptoms. We completed testing the validity of a shortened version of this existing questionnaire on patients referred to the Massachusetts Respiratory Hospital, and controls recruited by participating patients. This shortened questionnaire has been incorporated into the ongoing study survey.

EXPECTED PRODUCTS (MILESTONES): To develop a shorter questionnaire than the one currently used to identify MCS, scientific Publications on Persian Gulf War illnesses (including the impact of stress and trauma on outcome, annual reports, gender models of health outcomes.

STATUS/RESULTS TO DATE: A manuscript reporting the development of a short MCS questionnaire is currently under review (Hu, Stern, Rotnitzky et al.). After 3 mailings of the Time 4 questionnaire, approximately 43% of the original Devens cohort of 2949 PGW veterans have returned completed surveys. Additional phone surveys are planned to sample non-responders and assess the extent to which they may differ from responders. Appropriate weighting

procedures can then be applied to statistically minimize the effects of any response bias. Funding for this project ended in 1998. A final report containing some descriptive results was submitted to DoD in September 1998. Further analysis and manuscript preparations are on-going at this time.

PUBLICATIONS: Wolfe J, Proctor S, Davis JD, Sullivan M, Friedman M. (1998) Health symptoms reported by Gulf War veterans two years after return. *American Journal of Industrial Medicine*, 33, 104 -113, 1998.

Wolfe J, Sharkansky EJ, Read J, Dawson R, Martin J, Ouimette PC. Sexual harassment and assault as predictors of PTSD symptomatology among U.S. Persian Gulf War personnel. *Journal of Interpersonal Violence*, (13)40-57, 1998.

Proctor SP, Herren T, White RF, Wolfe J, Borgos, MS, Davis JD, Pepper L, Clapp R, Sutker PB, Vasterling JJ, Ozonoff D. Health status of Persian Gulf War veteran and: Self-reported symptoms, environmental exposures, and the effect of stress. *International Journal of Epidemiology*, (in press).

Wolfe J, Erickson DJ, Sharkansky EJ, King LA, King DW. Course and predictors of PTSD among Gulf War veterans: A prospective analysis. *Journal of Consulting and Clinical Psychology*, (in press).

Hu H, Stern A, Rotnitzky A, Schlesinger L, Proctor SP, Wolfe J. Development of a Brief Questionnaire for Screening for Multiple Chemical Sensitivity Syndrome. Manuscript submitted for publication, 1998.

Proctor SP, Wolfe J, Friedman M. Evaluation of health symptoms reported by Gulf War veterans. Paper presented at the Annual Meeting of the International Society for Environmental Epidemiology, Research Triangle Park, NC. September 1994.

Wolfe J, Brown PJ, Bucsela ML. Symptom responses of female Vietnam veterans to Operation Desert Storm. *American Journal of Psychiatry*, 149, 676-679, 1992.

Wolfe J, Brown P, Kelley J. Reassessing war stress: exposure and the Gulf War. *Journal of Social Issues*, 1993.

Title: Long-Term Effects of Subclinical Exposures to Sarin

Project #: DoD-53

Agency: DoD

Study Location: Albuquerque, NM

Project Status: Ongoing

Research Type: Mechanistic

P.I.: Rogene Henderson, Ph.D.

Research Focus: Chemical Weapons, Brain & Nervous System

Start Date (CY): 1997

Est. Completion (CY): 2000

OVERALL PROJECT OBJECTIVE: The Overall Project objective is to determine the toxicokinetics of inhaled sarin on F344 rats and to determine if single or repeated (5X and 10X) exposures to low levels of sarin results in changes in the quantity of cholinergic synaptic markers.

SPECIFIC AIMS: 1. Determine the dosimetry of inhaled tritiated-sarin. 2. Determine the effect of subclinical exposures to sarin on cholinergic synaptic markers.

METHODOLOGY: Specific Aim I will be accomplished in 3 steps: Step 1: Expose rats by inhalation to two levels of tritiated sarin; Step 2. Analyze brain slices and critical areas of the brain for tritium at three time points after exposure; Step 3. Determine clearance time for sarin and its metabolites.

Specific Aim II will be accomplished in 2 steps: Step 1. Expose rats to a high and a low dose of sarin; Step 2. Determine muscarinic and nicotinic receptor densities as well as acetylcholine esterase and choline acetyl transferase activities and choline uptake mechanisms in the brains of exposed rats at one (1) day and one (1) month after exposure. Results will illustrate the degree of persistence of the effects.

EXPECTED PRODUCTS (MILESTONES): In this study we expect to determine the dosimetry to the brain of different inhalation exposure concentrations of sarin and how long the agent remains in critical areas of the brain. We expect to complete these studies during the first 18 months of the study. We expect to complete the studies on the effect of single and repeated exposures to sarin on the quantity of cholinergic synaptic markers during the last year of the study.

STATUS/RESULTS TO DATE: The lab has been set up to meet safety and security requirements. The synthesis of 3H-sarin binary proved difficult and we recommend purchase of 3H-DDF, the de novo precursor to sarin instead. The exposure system was completed and the Minicam monitoring system set up. The conditions necessary to induce heat stress were determined and the technique for measurement of brain cell cytokines and apoptosis were developed. The first exposures are planned for January 1999.

PUBLICATIONS: Henderson RP, Dahl AR, Krone JR, Barr EB. Dosimetry of inhaled Sarin. Abstract presented at the Conference on Federally Sponsored Gulf War Veterans' Illnesses Research Meeting, Pentagon City, 1998,

Title: Assessment of Subchronic Neurobehavioral and Neuropathologic Effects in Rats

Project #: DoD-54

Agency: DoD

Study Location: Battelle, Columbus, OH

Project Status: Ongoing

Research Type: Mechanistic

P.I.: Carl Olson, Ph.D.

Research Focus: Interactions, Brain & Nervous System, Chemical Weapons

Start Date (CY): 1997

Est. Completion (CY): 1999

OVERALL PROJECT OBJECTIVE: The primary objective is to determine if exposure to low levels of sarin (GB) create neurobehavioral or neuropathologic effects. Another objective is to assess the effect of low-level GB exposure on possible toxic effects produced by other chemicals, especially chemicals that have a mechanism of action similar to that of GB.

SPECIFIC AIMS: The specific aim is to determine if subtle abnormal neurobehavioral or neuropathologic effects are detected as a result of short term exposure to low levels of chemicals, or combinations of chemicals, to which U.S. servicemen were exposed in the Persian Gulf area. A total of 9 groups of rats, vehicle control; GB; PB/DEET; CPF; Positive control; GB+PB/DEET; GB+CPF; CPF+PB/DEET, and GB+CPF+PB/DEET, with 24 rats per group will be evaluated in stages. Results will be analyzed to determine if any effects are statistically significant.

METHODOLOGY: The study is designed to evaluate neurobehavioral and neuropathologic alterations that may result from exposure to GB alone and from exposure to GB and a combination of chemicals, PB, DEET, and CPF, at dose levels which, when given alone, may produce a measurable effect (e.g., blood acetylcholinesterase [AChE] inhibition) but do not produce observable signs of exposure. The test system is a mature, outbred rat strain which is capable of exhibiting neuropathologic alteration following exposure to organophosphorous compounds known to cause delayed neuropathy. Pilot Studies are conducted to establish a dose of each chemical, given over a 4 day period, that may produce a measurable effect but does not produce clinical signs of intoxication. These doses will be used in the Subchronic Study in which animals are exposed to chemicals, as well as chemical combinations, over a 4 day period and monitored for up to 30 days. Neurobehavioral assessments (Functional Observational Battery [FOB] and evaluation of motor activity) are performed prior to and three times following exposures. At the time of sacrifice (30 days post-exposure), specimens from the central and peripheral nervous system are taken for neuropathologic evaluation. During both phases, blood AChE activities are assessed. A control compound, such as carbaryl, is used to produce neurobehavioral abnormalities.

EXPECTED PRODUCTS (MILESTONES): The milestones will be completion of the Pilot Studies, completion of the Subchronic Study neurobehavioral evaluations, completion of the neuropathology evaluations, statistical analyses of the data, and report preparation.

STATUS/RESULTS TO DATE: The contract for this proposal was signed September 29, 1997. MREF Protocol 134 was prepared and reviewed and approved by both Battelle's Institutional Animal Care and Use Committee (IACUC) and the U.S. Army MPMC Animal Use Review Officer, MAJ David L. Ruble. The use of GB already at the MREF was approved by USAMPMC. DEET and PB have been ordered and received, and 5 g of CPF were requested and received from Dow Elanco.

A shipment of 146 Sprague-Dawley rats approximately 3 months of age was received in December 1997. This is the oldest age at which rats are available, although a 6 month old rat is required for testing in subchronic studies. A technique for periodic blood sampling of these animals without the use of anesthesia is being developed. Rat blood is being analyzed for cholinesterase activity using both dithiobis(2-nitrobenzoic acid)(DTNB) with erythrocytes and dithionitrotic acid (DTNA) with whole blood in order to determine relationships and to attain better accuracy in measuring low levels of cholinesterase inhibition.

The pilot study of this task has been completed. A method to consistently obtain blood samples of 0.5 mL or greater from unanesthetized rats was attained and a number of staff trained in the procedure. A method for the analysis of whole blood ChE activity, using an automated Ellman procedure but with dithionitrotic acid (DTNA) rather than dithiobis (2-nitrobenzoic acid) as the analytical chromogen in order to prevent color interference by hemoglobin and to attain better replicability in the analyses, has been optimized and the technique documented and validated by Dr. James Blank.

A percutaneous dose of a methanol solution of CPF necessary to produce approximately 5 - 10 percent whole blood ChE-I is approximately 0.15mg/kg b.i.d. for four days. A s.c. GB dose of 0.75 µg/kg s.i.d. for four days was estimated to produce approximately 5 - 10 percent blood ChE-I and was *Persian Gulf Veterans' Illnesses Research Database - Project No DoD-54* selected for use in the sub-chronic study. An i.m.PB dose of 50 µg/kg s.i.d. for four days was selected for use in the 30 day study. A 15 µL volume of DEET applied to the skin b.i.d. for four days produced only mild erythema in two of ten rats, and this volume will be used in the sub-chronic study. When used as a positive control in neurobehavioral studies, carbaryl will be given approximately 30 min prior to evaluation at 30mg/kg by gavage using a 6 mg/mL corn oil solution. Saline at 30 µL/kg will be used in negative control animals.

Six MREF technicians have demonstrated their proficiency in performing neurobehavioral assessments of rats, and have had this proficiency attested to by Mr. John Merrill. A schedule has been established for the procurement of rats, for performing neurobehavioral assessments, for doing animals and performing ChE-I analyses, and for performing sacrifices for determining neuropathology in sub-chronic 30 - day studies. The task will be accomplished in 12 runs, with 18 rats per run and two rats per run in each of the nine treatment groups. The first run commenced in October 1998.

PUBLICATIONS: At the request of the U.S. Army, the journal Drug and Chemical Toxicology agreed to publish a portion of the proceedings of the United States Army Medical Research and Materiel Command's Bioscience Review held May 31 - June 4, 1998.

Title: Low-Level Exposure to GB Vapor in Air: Diagnosis/Dosimetry, Lowest Observable Effect Levels, Performance-Incapacitation, and Possible Delayed Effects

Project #: DoD-55

Agency: DoD

Study Location: Prinz Maurits Lab, Netherlands

Project Status: Ongoing

Research Type: Mechanistic

P.I.: Herman vanHelden, Ph.D.

Research Focus: Interactions, Chemical Weapons, Pyridostigmine Bromide

Start Date (CY): 1997

Est. Completion (CY): 2000

OVERALL PROJECT OBJECTIVE: To investigate the relationship between C.t-values, internal dose and adverse effects on performance in guinea pigs and marmoset monkeys which will be exposed to low levels of sarin (GB).

SPECIFIC AIMS: (1) At which C.t-value (t £ 5 h) of GB exposure does an internal dose of GB become measurable, i.e., what is the lowest observable effect level of exposure (LOEL)?; (2) At which C.t-value (t £ 5 h) of GB exposure and internal dose do these (systemic) effects of exposure start to have adverse effects on the performance of military personnel (LOAEL)?; (3) What are the consequences of continuous carbamate pretreatment during low level exposure to GB, i.e., will unexpected adverse effects on performance emerge through this combination of two cholinesterase inhibitors?; (4) What is the time course of adverse effects on performance following GB exposure and are there delayed effects?

METHODOLOGY: A number of central nervous system effects and miosis will be measured as sensitive and relevant parameters of incapacitation.

EXPECTED PRODUCTS: In this study we expect to get answers to the above-mentioned practically relevant questions.

STATUS/RESULTS TO DATE: A number of Technical Objectives are being conducted which are necessary to be able to execute the proposed experiments: (1) A glass exposure chamber for whole-body exposure of unanesthetized guinea pigs and marmosets (one animal per chamber) to low levels of GB vapor in air, has been designed and constructed, which is at the implementation stage. This equipment should meet a number of requirements in order to measure a number of parameters on-line during exposure in a safe way: respiration, EEG and miosis. Moreover, blood samples for toxicokinetic analysis and measuring ChE-activities should be taken during exposure; (2) The implementation of the telemetric method for measuring EEG-signals and the VER (visual evoked response) signal is making progress; (3) Different types of cameras have been tested for real-time measurement of miosis on both eyes in guinea pigs from outside the exposure chamber. The most suitable digital camera has been chosen; (4) The dosimetry method to determine the internal dose of GB bound to BuChE in guinea pigs is operational by now.

PUBLICATIONS: none to date

Title: Low-Level Sarin Neurotoxicity and Its Modulation by Pyridostigmine

Project #: DoD-56

Agency: DoD

Study Location: U of CA

Project Status: Ongoing

Research Type: Mechanistic

P.I.: Barry Wilson, Ph.D.

Research Focus: Interactions, Chemical Weapons, Pyridostigmine Bromide

Start Date (CY): 1997

Est. Completion (CY): 2001

OVERALL PROJECT OBJECTIVE: To "Conduct animal studies designed to assess the possible long-term or delayed clinical effects of low-level or subclinical exposures to chemical warfare agents." The nerve agent sarin (GB) will be assessed for its ability to induce peripheral neuropathy and myopathy in the presence and absence of co-exposure to pyridostigmine bromide (PB). Sensitive morphological methods will be used to examine the delayed effects of low-level and sub-clinical exposures to sarin on the structure of the hen and mouse brain, spinal cord, peripheral neuromuscular and peripheral sensory system.

SPECIFIC AIMS: Task one: Baseline DATA 1) Determine baseline levels (in the hen and mouse) of acetylcholinesterase (AChE), butyrylcholinesterase (BChE), and Neurotoxic Target Esterase

(NTE) in brain, RBC and plasma (the avian erythrocyte lacks AChE activity): AChE and BChE in the pectoral muscle; creatine kinase (CK) in blood. Establish the kinetics of inhibition of blood AChE/BChE to the test agents sarin, DFP, paraoxon and PB in vitro. Determine a high, non-lethal dosage and dosages that correspond to selected levels (i.e. 10%, 25%, 50%) of blood and brain AChE inhibition. Determine the differential agent sensitivity of hens and mice, and the induction of axonal degeneration in the spinal cord and peripheral nerves of DFP-treated mice. 2) Determine whether a single large dose of sarin (versus DFP and parathion) induces neurobehavioral signs of AChE-induced myopathy (e.g. proximal limb weakness and histopathology) and subsequent signs of OPIDN (distal weakness), and to correlate these findings with biochemical and morphological indices of nerve-fiber damage. Task two: Multiple Exposures - Assess the effects of multiple sarin exposures using a strategy of stepping down the dosages of sarin progressively until no effects are detected upon morphological assay of spinal cord, peripheral nerve and muscle of hens, and these tissues plus brain regions and sensory and motor terminals in mice. Task three: Pyridostigmine Bromide (PB) - Establish the highest no-effect dosage (HNED) of multiple doses of PB for use in Task Four. Experiments outlined in Task One and Task Two will be repeated, except that PB will be substituted for sarin. A single large dose of PB, and multiple smaller doses of PB, are expected to induce sub-junctional myopathy in vulnerable muscles. Absence of myopathic changes in animals treated with multiple doses of PB will provide the end-point for determination of the highest no-effect dosage of PB. Task four: Combined Effects of Sarin and PB - The final task is to determine whether the HNED of PB administered some days before, or shortly after sarin administration, causes the HNED of sarin to induce changes in biochemical or morphological parameters not present or greater than those found in animals treated with the same dose of sarin alone.

METHODOLOGY: The delayed effects of exposure to sub-lethal doses of sarin may include changes in: (1) brain; (2) muscle and spontaneous activity; (3) peripheral nerves/spinal cord and spontaneous activity. Compounds known to induce specific toxicities will be used as positive controls for #2 (parathion) and #1 and #3 (DFP). White Leghorn laying hens and male Swiss albino mice will be used as the test systems.

Biochemical determinations will be made using standard accepted methods. Cytochemical examination of muscle fibers will be carried out on frozen sections with routine histological techniques. Morphological and morphometric analyses will be made on tissue sample from perfused animals. Samples will be obtained from the following areas: frontal cortex, hippocampus, basal ganglia, cerebellum, medulla oblongata, mid-lumbar spinal cord, lumbosacral roots and dorsal root ganglia, proximal, mid-level and distal regions of nerves, and terminal-rich regions of sampled muscles. Tissues will be fixed and embedded in epoxy resin. One-micrometer-thick sections will be stained and examined by bright-field microscopy. Thin sections will be prepared as needed from sensory and motor nerve terminals, treated and examined with a transmission electron microscope. Morphometric assessment will be carried out on perfect cross-sections of identical regions of distal nerves that show the earliest changes of DFP neuropathy.

EXPECTED PRODUCTS (MILESTONES): Task One (years 1,2) will consist of "scoping" trials to establish appropriate dose/response ranges for sarin, DFP and paraoxon. Task Two (year 1,2) will determine thresholds and relative dose-effect levels for biochemical and morphological end-points. Tasks Three and Four (year 3) will examine whether pyridostigmine bromide (PB) induces responses to sub-threshold doses of sarin or DFP when the carbamate is given days before administration of the organophosphate (OP). The results will indicate how much and how often exposure to sarin is needed to produce sub-clinical (i.e. structural and functional) neurotoxic effects, which regions of the nervous system are most sensitive to sarin, and whether PB is able to modulate (increase/decrease) the neurotoxic potency of sarin.

STATUS/RESULTS TO DATE: Facilities are ready for nerve agent work. Monitoring procedures and US Army approvals are finalized, and preparations are underway to receive agent. Pilot experiments have been carried out using the OPIDN positive control DFP (1.5 mg/kg sc) and the AChE-induced myopathy positive control paraoxon (0.4 mg/kg sc). There were no neuropathological changes between the two treatment groups and saline controls. Experiments with these control chemicals continue while we wait for shipment of sarin.

PUBLICATIONS: Wilson BW, Henderson JD, Spencer PS. Clinical Effects of Low-level Exposures to Chemical Warfare Agents in Mice and Chickens. Drug and Chemical Toxicology (in press).

Low level sarin neurotoxicity and its modulation by pyridostigmine. Presentation at the USAMRMC 1998 Medical Defense Bioscience Review Program. Research for the Warfighter, May 31 - June 4, 1998.

Title: Physiologic Effects of Stress in Gulf War Veterans

Project #: DoD-57

Agency: DoD

Study Location: Georgetown Univ., Wash DC

Project Status: Ongoing

Research Type: Clinical Research

P.I.: Daniel Clauw, M.D.

Research Focus: Symptoms/General Health, Brain & Nervous System

Start Date (CY): 1997

Est. Completion (CY): 2000

OVERALL PROJECT OBJECTIVE: To address the association between Persian Gulf War Illness (PGWI) and biological stress responses among women who served in the Persian Gulf War (PGW), to determine if individuals with PGWI have responses similar to that of individuals with fibromyalgia and Chronic Fatigue Syndrome, specifically targeting the causal relationship between PGWI and stress.

SPECIFIC AIMS: 1. To perform clinical and psychological evaluation on a sampling of symptomatic and asymptomatic individuals deployed to the PGW; 2. To determine if PGWI patients show evidence of a low central corticotropin releasing hormone (CRH) state by administering IL-6 and evaluating ACTH response; 3. To demonstrate that PGWI patients show evidence of impaired activation of the adrenomedullary and sympathoneural components of the sympathetic nervous system evidenced by biological responses to dolorimeter testing, tilt table testing and computer testing; 4. To demonstrate that PGWI patients have evidence of a decreased peripheral responsiveness to catecholamines as seen in biological responses to tilt table testing; 5. To show that significant abnormalities to these components of the stress response can be noted in most PGWI patients and that the nature of the abnormality can predict the predominant clinical symptoms.

METHODOLOGY: The first year of research involved finalizing project plans, developing a sampling frame, and recruiting and evaluating participants. Patient recruitment will continue into the third year to include 120 symptomatic and asymptomatic subjects. Correlation will be made between self-reported medical information and, clinical and physiologic data. Data from this study will be linked to the Persian Gulf Women's Health Linkage Study for further analysis.

EXPECTED PRODUCTS (MILESTONES): To gain a better understanding of the pathophysiology of PGWI in order to improve recommendations for treatment of patients.

STATUS/RESULTS TO DATE: The final stages of project planning are currently underway and the early stages of patient recruitment have begun.

PUBLICATIONS: none to

Title: Illness Among Persian Gulf War Veterans: Case Validation Studies

Project #: DoD-58

Agency: DoD

Study Location: Univ of Iowa

Project Status: Ongoing

Research Type: Clinical Research

P.I.: Bradley Doebbeling, M.D.

Research Focus: Symptoms/General Health, Brain & Nervous System

Start Date (CY): 1997

Est. Completion (CY): 2001

OVERALL PROJECT OBJECTIVE: To compare the true rate of confirmed illness among samples of veterans deployed to the Gulf with and without these predefined conditions, versus true rate of confirmed disease among samples of veterans not deployed, with and without self-reported symptoms of these conditions.

SPECIFIC AIMS: The purpose of the current project is to compare the rates of false positive reports of illness among both deployed and non-deployed veterans. Because of the magnitude of the difference in prevalence between these groups, we feel it is important to explore and characterize their cognitive deficits, depression, and fibromyalgia. These studies will be performed by comparing the true rate of confirmed illness among samples of veterans deployed to the Gulf with and without self-reported symptoms of these conditions versus the true rate of confirmed illness among samples of veterans not deployed with and without these self-reported conditions. Furthermore we plan to also compare risk factors including medical and family history, psychological factors (such as major lifetime events or stress, personality traits, and social support), and occupational and environmental exposures for validated illness in a series of nested case-control studies for each illness outcome.

METHODOLOGY: A sample of veterans deployed to the Gulf and of non-deployed veterans who met pre-defined criteria for one of the three conditions of interest will be invited to participate in the follow-up studies. Additionally, a sample of subjects who did not meet criteria for any of the three conditions selected from among those participating in the telephone survey and deployed and not deployed to the Gulf will serve as controls. Selected subjects will be invited to participate in detailed personal interviews, physical examinations, risk factor assessment, structured neuropsychological, neurophysiological, psychiatric, and other laboratory testing. STUDY 1 is comprised of various tests for cognitive dysfunction. STUDY 2 is comprised of specific psychiatric testing and psychological assessment for depression. STUDY 3 is comprised of testing examining general health status for fibromyalgia.

EXPECTED PRODUCTS (MILESTONES): In this study, we plan to determine if the rate of false positives of the deployed Iowa Persian Gulf Veterans meeting a priori telephone survey criteria for cognitive dysfunction (CD) are the same as the rate of false positives of the nondeployed PGW veterans meeting the same criteria. We will also determine if the rate of false positives of the deployed PGW veterans meeting a priori criteria for depression are the same as the rate of false positives of the nondeployed PGW veterans. Finally we will determine if the rate of false positives of the deployed PGW veterans meeting a priori criteria for fibromyalgia are the same as the rate of false positives of the nondeployed PGW veterans meeting criteria. Furthermore, we expect to perform nested case-control studies of risk factors for illness among confirmed cases of illness.

STATUS/RESULTS TO DATE: September 25, 1998 marked the end of Year 1 of the grant. Further development of the study design, instrument selection and development, and project staffing are nearly complete. Pilot testing should begin by early December. Recruitment and assessment of research subjects will begin immediately thereafter. We have identified the pool of subjects eligible to participate in the project, and databases are being developed to allow management of study subject contact and manage the project's data.

PUBLICATIONS: Schwartz DA, Doebbeling BN, Merchant JA, et al. Self-reported illness and health status among Gulf War veterans: A population-based study. JAMA, 277:238-45, 1997. Crispino MR, Goerdts C, Burmeister LF, Doebbeling BN. Evaluation of health-related quality of life in Persian Gulf War Veterans. J Gen Intern Med, 10(4) (Supplement):41, 1995. Doebbeling BN, Rohrer JE, Woolson RF, Torner JC, Saag KG, Merchant JA, Bentler S, Schwartz DA. Health services utilization among Persian Gulf War and activated non-deployed veterans. Annual Meeting of the American Public Health Association. Indianapolis. IN. (poster)

November 11, 1997

Title: Pyridostigmine-induced Neurodegeneration: Role of neuronal Apoptosis

Project #: DoD-59

Agency: DoD

Study Location: Purdue Res Foundation

Project Status: Ongoing

Research Type: Mechanistic

P.I.: Gary E. Isom, Ph.D.

Research Focus: Pyridostigmine Bromide, Brain & Nervous System

Start Date (CY):

Est. Completion (CY): 2000

OVERALL PROJECT OBJECTIVE: Characterize the neurotoxic response to pyridostigmine by determining the exposure conditions required to induce neurodegeneration in the rat brain and examine the underlying mechanism in a neuronal cell model. This will be accomplished by administering varying doses of pyridostigmine to rats and at specific times, the brains will be examined histologically and by molecular biology procedures for apoptotic cell death. Cultured cells from the brain area(s) that undergo degeneration will be used to determine the molecular processes that pyridostigmine activates to initiate the brain cell death. Another objective is to determine the involvement of neuronal oxidative stress in the response and to determine if antioxidants will attenuate the degeneration in animals and cultured cell models.

SPECIFIC AIMS: 1. Characterize the dose-response of pyridostigmine-induced neuronal apoptosis in the rat brain and the ability of antioxidants to alter the neurotoxicity; 2. Detailed biochemical and molecular analysis of pyridostigmine-induced apoptosis in cultured cerebellar granule cells; 3. Determine the mechanism by which pyridostigmine stimulates the generation of intracellular reactive oxygen species and the linkage to apoptosis.

METHODOLOGY: Apoptosis will be documented in brain areas by the Apoptage (TUNEL) method, electron microscopy morphometric analysis and DNA (fragmentation) laddering on gel electrophoresis. Mechanistic studies will include measurement of redox transcription activation by EMSA, reactive oxygen species generation by microfluorescence procedures, and mitochondrial dysfunction by use of fluorescence dyes.

EXPECTED PRODUCTS (MILESTONES): In this study, we expect to show that pyridostigmine induces neurodegeneration by initiating apoptosis cell death in the brain. The mechanism of this brain injury will determine by use of neuronal cell models.

STATUS/RESULTS TO DATE: Apoptotic cell death in rat brain cortex, striatum, and hippocampus was noted after injection of pyridostigmine bromide (.25-1.85 mg/kg ip twice daily for 4 days). Lesions in the striatum and hippocampus were obtained only with higher doses. Cortical damage was evident even when rats were sacrificed 5-30 days after cessation of dosing indicating a continued cell destruction. Pretreatment with an antioxidant failed to protect against pyridostigmine-induced damage but atropine blocked the apoptosis caused by pyridostigmine.

PUBLICATIONS: Li L, Gunasekar PG, Borowitz JL, Isom GE. Pyridostigmine-induced acute and delayed neuronal apoptosis. Soc Toxicol Abstract, 1999.

Li L, Gunasekar PG, Borowitz JL, Isom GE. Pyridostigmine-induced neuronal apoptosis. (submitted for publication).

Title: Butyrylcholinesterase Genetic Variants in Persons with Gulf War Illness

Project #: DoD-60

Agency: DoD

Study Location: Univ of Nebraska Med Center

Project Status: Ongoing

Research Type: Clinical Research

P.I.: Oksana Lockridge, Ph.D.

Research Focus: Chemical Weapons, Pyridostigmine Bromide, Prevention

Start Date (CY): 1997

Est. Completion (CY): 1999

OVERALL PROJECT OBJECTIVE: Our goal is to test the hypothesis that some Gulf War illness may be caused by chemical damage to the nerves. If this hypothesis is correct we expect to find a correlation between low levels of butyrylcholinesterase or between genetically abnormal butyrylcholinesterase and Gulf War illness. Butyrylcholinesterase is a scavenger of nerve agents. A person with low levels of this enzyme or with a genetically defective enzyme would be less protected from nerve agents with the result that more of the agent would reach the nerves.

SPECIFIC AIMS: 1. To obtain blood samples from healthy and sick Gulf War veterans; 2. To obtain histories from the subjects detailing their exposure and symptom; 3. To measure the activity levels and phenotype of butyrylcholinesterase; 4. To determine the genotype of the butyrylcholinesterase; 5. To see if Gulf War Illness correlates with butyrylcholinesterase activity level or genotype.

METHODOLOGY: Gulf War veterans were recruited from the Omaha area. In addition, blood samples have been obtained from the VA Medical Center in East Orange, NJ. Histories were obtained by interviewing the subjects. Blood samples were tested for butyrylcholinesterase activity and phenotype with a spectrophotometric assay. The genotype was determined by amplifying the butyrylcholinesterase gene and sequencing the DNA.

EXPECTED PRODUCTS (MILESTONES): We have analyzed blood and DNA samples from 226 Gulf War veterans.

STATUS/RESULTS TO DATE: We tested the frequency of butyrylcholinesterase genetic variants in 226 veterans. 74 said they did not have Gulf War illness; 61 said maybe but they did not know; and 93 said they were sick with Gulf War illness. The frequency of genetic variants in the healthy group was 17.5%; in the maybe group was 21.0%; and in the sick group was 24.0%. The healthy group had 1 Fluoride allele and 25 K alleles; the maybe group had 2 Atypical alleles and 28 K alleles; and the sick group had 5 Atypical alleles and 40 K alleles. Thus, the sick group had a higher frequency of two low activity genetic variants of butyrylcholinesterase compared to healthy Gulf War veterans.

When these frequencies were compared to the frequency of butyrylcholinesterase genetic variants in the literature, it was apparent that our Gulf War veterans have a lower frequency of genetic variants than the 100,000 Europeans and Americans in the literature. The average American is expected to have a 1 in 25 chance of carrying the atypical allele, and a 1 in 4 chance of carrying the K variant allele, yet even our sick veterans had a lower frequency (1 in 37 for the atypical and 1 in 4.7 for the K variant allele). These results suggest that even the sick Gulf War veterans have a genetic makeup that makes them more resistant to organophosphates than the average non-military American. The statistical significance of the higher frequency of butyrylcholinesterase genetic variants in the sick Gulf War veterans still needs to be evaluated.

PUBLICATIONS: none to date

Title: Neurophysiologic and Neuropathologic Effects in Monkeys of Low Level Exposures to Sarin, Pyridostigmine, Pesticides, and Botulinum Toxoid

Project #: DoD-61

Agency: DoD

Study Location: Battelle

Project Status: Ongoing

Research Type: Mechanistic

P.I.: Carl Olson, Ph.D.

Research Focus: Interactions, Chemical Weapons, Pyridostigmine Bromide

Start Date (CY): 1997

Est. Completion (CY): 2000

OVERALL PROJECT OBJECTIVE: The primary objective is to determine if exposure to low levels of GB creates subtle enzymatic, neurophysiologic or neuromuscular pathologic effects. Another objective is to assess the effect of low-level GB exposure on possible toxic effects produced by other chemicals, especially chemicals that have a mechanism of action similar to that of GB.

SPECIFIC AIMS: To determine if subtle abnormal enzymatic, neurophysiologic or neuromuscular effects or neuropathology are caused as a result of short term exposure to low levels of chemicals, or combinations of chemicals, to which U.S. servicemen were exposed in the Persian Gulf area. A total of 5 groups of rhesus monkeys, a saline control; a positive control, GB/BotTox, PB/DEET/CPF; GB/BotTox+PB/Deet/CPF, with 8 monkeys per group are evaluated in two stages. Results are analyzed to determine if any effects are statistically significant.

METHODOLOGY: The study is designed to evaluate enzymatic, neurophysiologic and neuropathologic alterations that may result from exposure to GB/BotTox alone and from exposures to GB/BotTox and a combination of PB, DEET, and CPF, at dose levels which, when given alone, may produce a measurable effect (e.g., blood acetylcholinesterase [AChE] inhibition) but do not produce observable signs of intoxication.

Pilot Studies are conducted to establish a dose of each chemical, given over a 14 day period, that may produce a measurable effect but not produce clinical signs. These doses are used in the Subchronic Study in which animals are exposed over a 14 day period and evaluated for up to 270 days. Periodic neurophysiologic and electromyographic examinations of monkeys are performed. In addition, tissue specimens are examined for pathology at study termination. During the study, blood AChE, neurotoxic esterase (NTE), creatine kinase (CK) and alanine aminotransferase (ALT) activities are assessed. A control compound, diisopropyl fluorophosphate (DFP), is used to produce neurophysiologic and neuropathologic abnormalities.

EXPECTED PRODUCTS (MILESTONES): The completion of the Pilot Studies, completion of the Subchronic Study neurophysiologic evaluations at 24 hr, at 60 days, at 90 days, at 180 days, and at 270 days, completion of the neuropathology evaluations, completion of the statistical analyses of the data, and completion of a final report.

STATUS/RESULTS TO DATE: The ten monkeys selected for pilot studies with osmotic pumps using various concentrations of PB have been dosed. The mean whole blood ChE-I of monkeys given 0.1 mg/kg/day was 13 percent (7 and 19 percent), and the mean ChE-I of monkeys given 0.15 mg/kg/day was 24 percent (16 and 32). The monkeys given 0.27 mg PB/kg/day both had a ChE-I of 29 percent, and those given 0.5 mg PB/kg/day both had a ChE-I of 32 percent. Monkeys given 0.6 mg PB/kg/day had a mean ChE-I of 43 percent (39 and 47). A dose of PB between 0.15 and 0.25 mg/kg/day will be used in the subchronic study.

Following washout of the PB, the same monkeys were dosed once a day with DFP to determine the effects on NTE, ALT and CK, and on neurophysiology and neuropathology. Animals were injected with DFP s.c. on Mondays, Wednesdays and Fridays for two weeks, and blood samples were taken prior to dosing and approximately one hr after the last dose. Two monkeys were given 0.05 mg/kg the first three days and 0.01 mg/kg the last three days. Another two monkeys were given 0.1 mg/kg the first day, 0.05 mg/kg at the second and third dosings, and 0.01 mg/kg the last three days. Doses were reduced due to vomiting, lethargy and anorexia. ChE-I was 92 percent in both of the higher dose animals, and a mean of 80 percent (76 and 83 percent) in the other two monkeys. ALT levels were not increased, CK levels were variable (65 to 302 percent of baseline), and NTE levels were reduced to 39 to 53 percent of baseline. Neurophysiology examinations were performed on these monkeys prior to DFP injection and again approximately 30 days after the start of DFP injections by Dr. Michael Podell of the Ohio State University (OSU). Dr. Zarife Sahenk of OSU's neurology clinic was at Battelle during the necropsy of the first monkey and demonstrated techniques to use to sample tissue for neuropathologic evaluation. The additional three monkeys dosed with DFP have been sacrificed and selected tissues harvested. The other six monkeys previously given PB have undergone neurophysiologic examinations, and dosing of these animals with DFP was started at the end of September.

Eight monkeys were dosed s.c. with GB three times a week for two weeks. Two were dosed at 2.5. two at 0.5. two at 0.25. and two at 0.1 mg/kg. Mean ChE-I values were 67 percent (56 and 77

percent), 18 percent (9 and 27 percent), 14 percent (7 and 20 percent), and 14 percent (13 and 14 percent), respectively. ALT values were not noticeably elevated and CK values again were extremely variable. NTE activity was virtually zero in the monkeys dosed at 2.5 mg/kg and in one of the monkeys dosed at 0.25 mg/kg. In the other monkeys, NTF ranged from 41 to 87 percent of baseline. An additional two monkeys will be dosed at 0.01 mg GB/kg starting early in October and the ten monkeys dosed with GB, following a washout period, will have various volumes of CPF in methanol solution applied to skin clipped of hair. This will be done twice a day, Monday through Friday, to determine a volume of CPF solution that produces a low but measurable level of whole blood ChE-I approximately one hr following the last dose on Friday morning of the second week. The six monkeys dosed with DFP will undergo their post-exposure neurophysiologic examinations by Dr. Podell the end of October, approximately 30 days following the start of DFP dosing. These six monkeys then will be sacrificed and tissues sampled for neuropathologic evaluations, using electron microscopy and nerve teasing procedures, by Dr. Sahenk. Following completion of pilot studies, doses of test compounds will be selected for use in the 9-month sub-chronic evaluation.

PUBLICATIONS: none to

Title: Sarin and Pyridostigmine Interaction under Physical Stress: Neurotoxic Effects in Mice

Project #: DoD-62

Agency: DoD

Study Location: S. Ill Univer

Project Status: Ongoing

Research Type: Mechanistic

P.I.: Satu Somani, Ph.D.

Research Focus: Interactions, Chemical Weapons, Pyridostigmine Bromide

Start Date (CY): 1997

Est. Completion (CY): 1999

OVERALL PROJECT OBJECTIVE: To elucidate whether physical stress amplifies the neurotoxic effects and neuromuscular abnormalities after administration of pyridostigmine, sarin and the combination of these two agents.

SPECIFIC AIMS: (i) To evaluate the neurotoxic effects of low dose sarin in mice using behavioral (muscular weakness, ataxia, and motor dysfunction), electrophysiological (neuromuscular function), biochemical (inhibition of cholinesterase, neurotoxicesterase (NTE), and enhanced lipid peroxidation) in brain, spinal cord, platelets, and sciatic nerve and histopathological (light and electron microscopy) (axonal degeneration and demyelination in spinal cord and sciatic nerve and neuromuscular damage) analyses. (ii) To compare the above neurotoxic effects induced by low dose sarin exposure in pyridostigmine treated mice with untreated mice so as to determine the possible interactive effects of pyridostigmine with sarin-induced neurotoxicity. (iii) To evaluate the effects of physical stress on low dose sarin, pyridostigmine, and the combination of two to determine the above parameters and correlate with morbidity associated with Gulf War veterans.

METHODOLOGY: This study was designed to investigate the interactive and delayed effects of pyridostigmine and exercise on behavioral, electrophysical, biochemical, and histopathological changes in peripheral and cerebral tissues of mice. Four groups of Male NIH Swiss mice were treated as follows: 1) sedentary control; 2) exercise training for ten weeks; 3) pyridostigmine (1.2 mg/kg, P.O.) daily for two weeks; and 4) pyridostigmine plus exercise training for ten weeks. The animals were observed for behavioral changes. The muscle tension was measured in both legs using a tension transduction device connected to a polygraph after the last dose of pyridostigmine or saline and 24 hr after exercise training. Mice were then sacrificed. Blood and tissues were isolated and analyzed.

EXPECTED PRODUCTS (MILESTONES): In this study we expect to determine that physical stress amplifies the chronic delayed effects of pyridostigmine in mice.

STATUS/RESULTS TO DATE: Results indicated a significant increase in the muscle tension of combined legs in Group 4. Butyrylcholinesterase and acetylcholinesterase activity significantly

decreased in plasma and triceps muscle respectively, in Group 4. Neurotoxic esterase activity significantly decreased in cerebral cortex in Group 2. Creatine phosphokinase activity increased 122% of control in plasma Group 4. Malondialdehyde concentration significantly increased in triceps muscle in Group 4 indicating the oxidative stress of the combination. Electron microscopy of spinal cord and sciatic nerve did not show ultrastructure changes among groups. The data suggests that the interactive and delayed effects of pyridostigmine (even after four weeks of stoppage of dosage) and exercise training occurred primarily in peripheral tissues. In conclusion, physical stress amplified the delayed muscular effects of pyridostigmine in mice.

PUBLICATIONS: Somani SM, Husain K, Scott B, Verhulst S, Reddy KS, Montrey R, Helfert R. Delayed and Interactive Effects of Pyridostigmine and Physical Stress on Biochemical Indices in Peripheral and cerebral Tissues of Mice. Experimental Biology 99, April 1999, Washington, DC.

Verma-Ahuja S, Husain K, Verhulst S, Espinosa JA, Somani SM. Delayed Effects of Pyridostigmine and Exercise Training on Muscle Tension in Mouse Lower Extremity. Experimental Biology 99, April 1999, Washington, DC.

Husain K, Somani SM. Influence of Physical Stress and Pyridostigmine on Cholinesterase Activity in Blood and Brain regions of Male and Female Mice. Experimental Biology 99, April 1999, Washington, DC.

Jagannathan R, Husain K, Reddy KS, Somani SM. Pyridostigmine and Exercise Interaction on cardiac Antioxidant Defense System in Mice. Society of Toxicology Meeting, New Orleans, March 14-18, 1999.

Title: PGW Veterans: Epidemiological and Clinical Evidence for Residual Organophosphate Neurotoxicity

Project #: DoD-63

Agency: DoD

Study Location: Oregon Health Sciences Univ

Project Status: Ongoing

Research Type: Clinical Research

P.I.: Peter Spencer, Ph.D.

Research Focus: Brain & Nervous System, Chemical Weapons

Start Date (CY): 1997

Est. Completion (CY): 2000

OVERALL PROJECT OBJECTIVE: We seek definitive evidence to support or refute the proposal that Gulf War veterans have nervous-system deficits consistent with prior exposure to organophosphorous (OP) chemicals. Focus is placed on Desert Storm soldiers who served in March 1991 within a 50-km radius of Khamisiyah, Coalition-Occupied Iraq, and who may have been exposed to airborne sarin/cyclosarin following detonation of Bunker #73, the site of a chemical-weapons cache that may also have included weaponized mustard.

SPECIFIC AIMS: 1. Compare the neurobehavioral and neurophysiological status of GW veterans who were present in the 50-km radius area of Khamisiyah during the first two weeks of March 1991 (study group) with that of (a) other Desert Storm veterans with no known potential exposure to nerve agents, (b) non-deployed GW-era veterans (negative controls) and (c) subjects with documented, symptomatic, non-convulsive exposure to an organophosphate (OP) such as sarin or an OP pesticide (positive controls). Search for evidence of persistent changes in the nervous system reportedly associated with symptomatic exposure to sarin or other OPs. Determine the potential of developing an inexpensive neurobehavioral-screening test to predict neurological damage associated with OP poisoning.

METHODOLOGY: Symptoms, functional neurobehavioral performance and neurophysiological measures will be assessed in study groups. The fit between the spatial-temporal location of veterans, known releases of OP nerve agents, and evidence of neurological deficits will be

determined.

EXPECTED PRODUCTS (MILESTONES): 1. Obtain a database from Department of Defense (DoD) of the DoD-estimated 20,000 individuals in the Khamisiyah cohort (50-km radius only) with possible sarin/cyclosarin exposure. Complete sampling plan. 2. Locate deployed and non-deployed subjects and carry out exposure/health interviews. 3. Characterize OP-related nervous system changes in civilian positive controls. 4. Carry out and analyze results of neurobehavioral testing (Level I testing). 5. Carry out and analyze results of neurophysiological examination (Level II testing).

STATUS/RESULTS TO DATE: 1. Received (2/98) Surgeon General's Human Subjects' Committee approval of study protocol, consent forms, and questionnaires. 2. Requested (2/98) and received (5-6/98) Defense Manpower Data Center data tapes containing identifiers of GW-era veterans originating from Oregon, Washington, or California. 3. Analyzed available subjects from targeted western states (too few for study needs) and extended study to North Carolina, Colorado and Georgia to obtain adequate sample of individuals for Khamisiyah cohort. 4. Completed (8/98) sampling plan for western states. 5. Initiated tracking, locating and CATI interviewing of deployed and non-deployed subjects; completed interviews on 160+ subjects. 6. Conducted pilot neurobehavioral testing (for Level I studies). 7. Conducted pilot neurophysiological examinations (for Level II studies). Sought assistance in identifying positive-control subjects from: (a) CBDCOM, for sarin-exposed subjects (7/98), and (b) western state governmental agencies, for OP-exposed civilians (1998) [responses pending].

PUBLICATIONS: Spencer PS, Schaumburg, HH, Ludolph AC. Experimental and Clinical Neurotoxicology, 2nd Edition. Oxford Univ Press, New York (in press).
Spencer and the Portland Environmental Hazards Research Center Team. Health effects of exposures in the Gulf War. VIIIth International Congress on Toxicology, Paris, France, July 1998, in press.
Spencer PS, McCauley LA, Joos SK, Lasarev MR, Shuell T, Bourdette D, Barkhuizen A, Hohnston W, Storzbach D, Wynn M, and other members of the Portland Environmental Hazards Research Center. Gulf War veterans: Service periods in theater, differential exposure, and persistent unexplained illness. Toxicology Letters (in press).
Anger WK, Storzbach D, Amler RW, Sizemore OJ. Human behavioral neurotoxicology: Workplace and community assessments. In Rom W, Environmental and Occupational Medicine, 3rd edition, Philadelphia, Lippincott-Raven pp. 709-731.
Gold BG, Schnell J, Spencer PS. Neurotoxic Disorders. In: Atlas of Neurology. (ed. Rosenberg RN), Butterworth-Heinemann, Philadelphia. PP 14.1-14.2.
Spencer PS, Wilson B, Albuquerque EX. Sarin, other "nerve agents" and their antidotes. In: Experimental and Clinical Neurotoxicology, (ed. Spencer, P.S. and Schaumburg, H.H.), Oxford University Press, New York, 1999, (in press).
Spencer PS, Daniels J, Kisby G. Mustard warfare agents and related substances. In: Experimental Clinical Neurotoxicology, (ed. Spencer, P.S. and Schaumburg, H.H.), Oxford University Press, New York, 1999, (in press).
Spencer PS, Anger WK, McCauley L, Esseks E. Perceived exposure to chemical weapons and unexplained illness among military and civilian subjects. 1998 USMRICD Medical Defense Bioscience Review, Maryland

Title: Individual Differences in Neurobehavioral Effects of Pyridostigmine

Project #: DoD-64

Agency: DoD

Study Location: Midwest Res Institute

Project Status: Ongoing

Research Type: Clinical Research

P.I.: Marv Cook. Ph.D.

Research Focus: Pyridostigmine Bromide, Brain & Nervous System, Prevention

Start Date (CY): 1997

Est. Completion (CY): 2001

OVERALL PROJECT OBJECTIVE: To provide the U.S. Army with a more complete body of knowledge for optimal use of pyridostigmine as a prophylactic organophosphate-defense agent if a future large-scale deployment is needed.

SPECIFIC AIMS: 1) Conduct a study to quantify any physiological or performance consequences of the use of pyridostigmine; 2) determine whether exposure to heat exacerbates any effects found; and, 3) evaluate individual differences in cholinesterase inhibition that might predict deleterious response to pyridostigmine.

METHODOLOGY: In the first study, two groups of subjects (at least 18 men and 18 women per group) will be randomly assigned to take 30 or 60 mg PYR for 13 doses at 8-hour intervals. Each subject will also take 13 doses of placebo, and order of PYR and placebo will be counter-balanced. Testing will occur on days 4 and 5 of each drug regimen. The test battery to be administered includes physiological, sensorimotor, and cognitive measures. In the second study, men and women will follow the same pyridostigmine regimen, but will be tested in the heat on those physiological and performance tasks that showed effects in the first study.

EXPECTED PRODUCTS (MILESTONES): Study 1 should be completed by December 1999.

STATUS/RESULTS TO DATE: Data collection for Study 1 began in July 1998.

PUBLICATIONS: none to

Title: Multi-disciplinary Pathophysiologic Studies of Neurotoxic Gulf War Related Syndromes Leading to Diagnosis and Treatment

Project #: DoD-65

Agency: DoD

Study Location: Univ of TX SW Med Cntr at Dallas

Project Status: Ongoing

Research Type: Clinical Research

P.I.: Robert Haley, M.D.

Research Focus: Treatment

Start Date (CY): 1994

Est. Completion (CY): 2000

OVERALL PROJECT OBJECTIVE: 1. Demonstrate the specific sites and nature of neurologic damage associated with the symptoms comprising the case definition, 2. Develop a two-stage system of testing and confirming the diagnosis of neurological impairment in Gulf War veterans for use in cost-effective screening of large populations of Gulf War veterans; 3. Provide insights into the pathophysiology of the neurologic impairment of Gulf War veterans upon which to base approaches to treatment.

SPECIFIC AIMS: 1. Extend the battery of tests for detecting neurotoxic brain and nerve damage and apply the new battery to the original 43 cases and controls (proposal to enlarge the sample was not funded by the Persian Gulf Veterans Coordination Board); 2. Plan and conduct a population survey of Gulf War veterans to estimate the prevalence of Haley's factor analysis-derived case definitions of Gulf War-related neurologic syndromes; 3. Apply the tests for neurotoxic brain and nerve damage to a new set of cases and controls selected randomly from the population survey.

METHODOLOGY: Tests to be applied to the cases and controls with investigators blinded to case-or control-group status include audiovestibular tests of brainstem function including high speed infrared oculography, scans of regional cerebral blood flow (HMPAO-SPECT) before and after a cholinergic challenge with physostigmine, magnetic resonance spectroscopy of the basal ganglia and brainstem to detect gliosis or neuronal loss, 24-hour Holter monitor with computer analysis of heart rate variation to assess parasympathetic nervous system function, direct recording of sympathetic nerve firing rate from the peroneal nerve by microneurography, quantitative EEG, sleep studies with polysomnography and measurement of circadian rhythm of

tyinpanic temperature, tests of peripheral blood neurohormone levels, genetic tests for paraoxonase and butyrylcholinesterase genotypes and blood enzyme levels, psychiatric interviews for stress-related and other psychopathology, batteries of neuropsychologic tests particularly focusing on tests of subcortical brain function, and evaluation of joint function with standard roentgenographic, MRI and proprioception studies. Planning of the national survey involves analyzing the characteristics of the deployed and nondeployed Gulf War-era military population, developing methods to avoid selection bias from the "healthy-warrior effect," translating Haley's self-administered survey questionnaires into computer-assisted telephone interview format, pretesting the survey method, and designing and selecting an efficient stratified random sample of deployed and nondeployed populations for survey.

EXPECTED PRODUCTS (MILESTONES): By the end of the first phase (March 1999), we expect to have demonstrated the extent and nature of neurotoxic damage responsible for symptoms satisfying Haley's case definitions of the Gulf War-related neurologic syndromes, have demonstrated the pathophysiologic basis for many of the symptoms and genetic predispositions for neurologic damage from organophosphate exposure, have identified a subset of tests most useful for identifying the syndromes, and have completed the design of a national random sample survey to estimate the prevalence of the syndrome in the deployed and nondeployed populations of Gulf War-era veterans. By the end of the second phase (December 1999), we expect to have demonstrated whether the veterans satisfying Haley case definitions in the population sample have the same neurologic basis as those in the initial sample.

STATUS/RESULTS TO DATE: 1. Conducted an epidemiologic survey of a reserve Seabees battalion, developed a case definition of Gulf War-related neurologic dysfunction (6 syndrome variants), demonstrated that cases satisfying the case definition had organic neurologic dysfunction compared with matched controls, conducted a further case-control analysis showing that the cases were more likely (RR 4-8) than controls to report wartime exposure to being present during a chemical weapons alarm, being present near Khafji on January 19 or 20, 1991, having advanced adverse reactions to pyridostigmine bromide, using large amounts of government-issue insect repellent, wearing pet tick and flea collars, and working as a security guard. These findings supported the theory that the Gulf War-related neurologic syndrome was caused by exposure to combinations of organophosphate-like chemicals in the war. 2. We designed experiments to test this theory in hens, carried them out collaboration with Duke and Kansas State Universities and EPA, and demonstrated that combinations of DEET, pyridostigmine, permethrin and chlorpyrifos act synergistically to cause delayed, chronic neurotoxicity. 3. Completed a study of the neuropsychologic evidence of organic brain dysfunction in ill Gulf War veterans. 4. Completed a study of the audiovestibular evidence of brainstem dysfunction in ill Gulf War veterans. 5. Conducted a literature review of 19 published reports of posttraumatic stress disorder in Gulf War veterans demonstrating that the apparent prevalence of PTSD was entirely due to errors in measurement and that there is no basis for the contention that Gulf War veterans are ill from PTSD and combat stress. 6. Reanalyzed the evidence in three epidemiologic studies comparing the prevalence of mortality, hospitalization and birth defects in deployed versus nondeployed Gulf War-era populations and demonstrated that the original conclusions of no war-attributable effects were due to bias from incorrect calculation of confidence intervals, selection bias from the "healthy-warrior effect," and selection bias from incomplete follow up of veterans who separated from the service. 7. Completed the pathophysiologic testing protocol on 40 of the 43 members of the original case-control study, and after completing the final three subjects will break the blinding code and begin the analysis. 8. Completed planning of the national random sample survey and are ready to begin the pretest.

PUBLICATIONS: Haley RW. Point: Bias from the "healthy warrior effect" and unequal followup in government studies of the health effects of the Gulf War. *Amer J Epidemiol*, 148:315-323, 1998. (Also included in *Amer J Epidemiol*, 148:324-338, 1998 are four other counter point articles.)

Haley RW. Is Gulf War syndrome due to stress? The evidence reexamined. *Am J Epidemiol*, 146:695-703. 1997. (See correspondence and author's replies *Am J Epidemiol*. 148:402-470.

1998).

Haley RW, Kurt TL. Self-reported exposure to neurotoxic chemical combinations in the Gulf War: a cross-sectional epidemiologic study. JAMA, 277:231-237, 1997.

Horn J, Haley RW, Kurt TL. Neuropsychological correlates of Gulf War syndrome. Arch Clin Neuropsychol, 12:531-544, 1997.

Haley RW, Kurt TM, Horn J. Is there a Gulf War syndrome? Searching for syndromes by factor analysis of symptoms. JAMA, 277:215-222, 1997.

Haley RW, Kurt TM, Roland PS, et al. Evaluation of neurologic function in Gulf War veterans: a blinded case-control study. JAMA, 277:223-230, 1997

Title: Testing for mycoplasmal infection replicability of nucleoprotein gene tracking and forensic polymerase chain reaction

Project #: DoD-66

Agency: DoD

Study Location: WRAIR Medical Center

Project Status: Ongoing

Research Type: Development

P.I.: Charles Engel, Jr., M.D.

Research Focus: Diagnosis

Start Date (CY): 1998

Est. Completion (CY): 1999

OVERALL PROJECT OBJECTIVE: To determine the reliability of three tests for Mycoplasma fermentans (incognitus strain) in human pure blood mononuclear cells, "usual" polymerase chain reaction procedure (UPCR), an optimized chelex polymerase chain reaction (OCPCR) procedure, and a nucleoprotein gene tracking (NGT) procedure.

SPECIFIC AIMS: 1) determine and compare the test-retest reliability of the usual PCR, optimized PCR, and NGT procedures at a laboratory experienced in these testing procedures; and 2) determine and compare inter-lab reliability of the usual PCR, optimized PCR, and NGT procedures at four different laboratories.

METHODOLOGY: 60 symptomatic Gulf War veterans (30 previously untested, 10 previously tested positive on UPCR, 10 previously tested negative on OCPCR, and 10 previously tested negative on NGT) will have blood drawn, aliquoted, and shipped overnight to 4 different laboratories for mycoplasmal testing. One of the labs, experienced in the 3 testing procedures, will retest each subject. Each aliquot will receive a separate identification code so that all labs are completely blinded as to what subject a given aliquot came from and whether any two aliquots are from the same subject. Cohen's Kappa will be used to determine the rate of agreement for each test adjusting for chance agreement.

EXPECTED PRODUCTS (MILESTONES): Information pertaining to the relative utility of these three mycoplasmal testing procedures. A manuscript summarizing the research will be submitted for peer-reviewed publication.

STATUS/RESULTS TO DATE: Participating labs were identified and received lab manuals and instruction in the testing procedures. Two series of five quality control blood bank blood samples were sent to all labs to ensure each lab could perform the tests at their own site. Minor adjustments were recently made to the protocol to allow for increased subject recruitment efforts when the previously expected subject source was unproductive.

PUBLICATIONS: none to date

Title: Antibacterial Treatment Method Based Upon the Excretion of Dead and Decaying Spherical Bacteria

Project #: DoD-67

Agency: DoD

Study Location: Louisiana Med Foundation

Project Status: Ongoing

Research Type: Clinical Research

P.I.: Edward Hyman, M.D.

Research Focus: Brain & Nervous System, Symptoms/General Health, Diagnosis

Start Date (CY): 1997

Est. Completion (CY): 1999

OVERALL PROJECT OBJECTIVE: To establish the relation of Desert Storm Syndrome (DSS) to Systemic Coccal Disease (SCD), to characterize the infectious agent(s) involved, and to evaluate the effectiveness of the diagnostic and treatment methods we have used in civilian SCD for the management of veterans with DSS, to train VA and/or DoD medical personnel in the use of the methods.

SPECIFIC AIMS: see objectives.

METHODOLOGY: The primary clinical study is a two arm, randomized, patient and evaluator blinded, placebo controlled, pilot study of an already-tested diagnostic/therapeutic regiment for the treatment of DSS.

The methods include: 1) demonstration of bacteremia by the urinalysis method of Dr. Hyman (ref. 1,2,3 in 13 vide infra); 2) randomization of patients to treatment or control group; 3) quantitative evaluation of the manifestations of DSS by a blinded evaluating team at S.U.N.Y.-Stony Brook; 4) suppression or elimination of bacteremia by antibiotic treatment, controlled and modified by continuing surveillance of cocciuria (ref 3); 5) re-evaluation of patients (treated or placebo treated) by the blinded team at S.U.N.Y.; 6) treatment of placebo group as in D; 7) re-evaluation of the now treated, previously placebo, patients.

EXPECTED PRODUCTS (MILESTONES): We expect to establish whether the treatment is or is not effective in improving fatigue, cognitive dysfunction, joint and somatic pain, headache, dermatitis, GI dysfunction. We expect to characterize the infecting organisms.

STATUS/RESULTS TO DATE: The code has not yet been broken. No results are available.

PUBLICATIONS: Hyman ES. Improved microscopic detection of bacteriuria. Biotech Histochem, (27)1:1-8, 1992. Hyman ES. A computer algorithm offers a comprehensive view of quantitative bacteriuria. Nephron, 65:549-558, 1993.

Hyman ES. A Urinary Marker for Occult Systemic Coccal Disease. Nephron, 68:314-326, 1994.

Title: Five Year Follow-Up of arm Personnel Exposed to Chemical Warfare Agents

Project #: DoD-69

Agency: DoD

Study Location: Institute of Medicine/Medical Follow-up Agency

Project Status: Ongoing

Research Type: Epidemiology Research

P.I.: Dr. Philip Renzullo

Research Focus: Chemical Weapons, Symptoms/General Health

Start Date (CY): 1997

Est. Completion (CY): 2000

OVERALL PROJECT OBJECTIVE: This epidemiological study of morbidity and mortality outcomes associated with exposure to potentially low levels of chemical warfare agents at Khamisiyah, Iraq is being performed at the request of the Department of the Army. Health outcomes of troops putatively exposed during rocket demolition in 1991 will be compared to those of a similar groups of unexposed military personnel for a five-year follow-up period. Exposure levels will be estimated based on environmental and climatological modeling of the chemical footprint, in combination with troop location data during the time of the demolition.

Specific objectives of the study are 1) to compare morbidity and mortality outcome rates among

Army personnel putatively exposed to chemical warfare agents and those not exposed using passive records-based methods; and 2) to compare temporal trends in health perception and health care use before and after notification of possible chemical warfare agent exposure among Army personnel putatively exposed and those not-exposed.

SPECIFIC AIMS: see objectives.

METHODOLOGY: The cohort for this study will be selected in collaboration with OSAGWI, the US Army Center for Health Promotion and Preventive Medicine (Deployment Environmental Surveillance Program), and the Department of Veterans Affairs (Environmental Epidemiology Service). Eligibility for entry into the cohort will be based on having served in to Persian Gulf Theater of Operations. Individuals identified as having been within and outside the modeled chemical footprint will be eligible for inclusion. The cohort will be defined not only in terms of potential chemical exposure, but also in terms of whether or not individuals were notified of potentially having been exposed to chemical agents, and whether or not individuals participated in the VA National Health Survey of Persian Gulf War Veterans. These three two-level exposure variables will result in 8 possible exposure groups for study.

EXPECTED PRODUCTS (MILESTONES): Findings and conclusions will be published jointly with DoD and VA investigators in a peer-reviewed professional journal.

STATUS/RESULTS TO DATE: none reported.

PUBLICATIONS: none to date

Title: War Syndromes from 1900 to the Present: Symptom Patterns and Long-term Health Outcomes

Project #: DoD-70

Agency: DoD

Study Location: Royal Defence Med. Coll., London

Project Status: Ongoing

Research Type: Epidemiology Research

P.I.: LTC Ian Palmer

Research Focus: Symptoms/General Health

Start Date (CY): Est. Completion (CY): 2000

OVERALL PROJECT OBJECTIVE: The primary hypothesis tested in this study, after corrections for societal influences and historical medical descriptors, is that symptom patterns and long-term health outcomes of war syndromes are consistent between the conflicts studied and over time.

SPECIFIC AIMS: The study comprises two parts. Part 1 is a retrospective comparison of symptoms characterized as "War Syndromes" following military service in the British and American armies from 1900 to the present via examination of primary source data from hospital and pension records. Part 2 will examine the long term morbidity and mortality of one of the syndromes.

METHODOLOGY: Because, historically, "War Syndromes" have been interpreted both on changing medical response and the societal influences on views of etiological factors, part 1 of the study will try to determine whether the symptomology of historical "War Syndromes" is similar and whether the labels appended to the symptom complexes can be reliably distinguished from each other by blinded statistical (factor) analysis. Records for comparison will be obtained from the United Kingdom (UK) Ministry of Defence's Medical Assessment Program (MAP); the United States' Comprehensive Clinical Evaluation Program (CCEP) data base; and abstracted records from data sources identified from 1900 to the present (UK's Public Record Office and War Pension Agency and U.S. Department of Social Security and Military Hospital Records amongst others). In part 2 an examination of risk factors, morbidity, mortality and changes over time in the explanations or attributions (self-reported or from medical personnel diagnosis) will be accomplished. This will be achieved in three ways: 1) a case control-study of Effort Syndrome/Disordered Action of the Heart (DAH)/Shell-shock will be compared with a matched group of soldiers receiving a pension for loss of a single limb; (2) an historical narrative analysis of morbidity will be performed; and (3) a retrospective cohort analysis will be performed on

mortality data. It is anticipated that the two part project will allow for a broader understanding of the factors involved in the medical description and societal negotiation of a "War Syndrome" following active service in the Gulf War of 1990/1991.

EXPECTED PRODUCTS (MILESTONES): This understanding and accumulated project data will, in turn, provide a basis for appropriate interventions, research and compensatory provisions in the management of current and future "War Syndrome" patients.

STATUS/RESULTS TO DATE: none reported.

PUBLICATIONS: none to date

Title: A Comparison of Post Deployment Hospitalization Between Vietnam and Gulf War Veterans

Project #: DoD-71

Agency: DoD

Study Location: NHRC

Project Status: Ongoing

Research Type: Epidemiology Research

P.I.: Christopher Blood, M.A.

Research Focus: Symptoms/General Health

Start Date (CY):

Est. Completion (CY): 2000

OVERALL PROJECT OBJECTIVE: This study will examine the role of exposure to war generally on the health problems of Gulf War Veterans (GWVs) by comparing post-deployment hospitalization rates of GWVs with post-deployment hospitalization incidence of Vietnam veterans.

SPECIFIC AIMS: The study will attempt to determine: if the relationship between the health problems of some veterans and their deployment to the Gulf War theater of operations is unique to the Gulf War or whether there is a relationship between certain health problems and exposure to war's combat environment generally, rather than exposure to a specific etiologic agent within that environment.

METHODOLOGY: The investigation, a statistics oriented comparison of incidence rates among two populations, will examine this possibility by contrasting the post-deployment hospitalization incidence of GWV with the hospitalization incidence of veterans of the Vietnam conflict. Controls for the comparison populations are provided by matching, as closely as possible, the varying types of Marine Corps units deployed to both combat theaters of operations (infantry units vs. support units Vs service support units). The study populations from the Vietnam War, all serving at that conflicts very conclusion, include 1) infantry battalions of the 1st Marine Division, 2) battalions of the 11th Marine Artillery Regiment, the 1st Reconnaissance Battalion, and the 1st Engineer battalion, and 3) the headquarters battalion, supply battalion, and maintenance battalion of the 1st Force Service Regiment. For the Gulf War, infantry battalions from the 1st Marine Division will also be used as the combat unit study population, personnel from two battalions of the 11th Marines combined with the 1st Reconnaissance Battalion, and the 1st Combat Engineer Battalion will form the combat support group, and the Marines attached to the headquarters battalion, maintenance battalion and supply battalion of the First Force Service Support Group (FSSG) will compose the service support group. Analyses will examine hospitalization incidence by person-years across a 5 year period following the conflicts, using International Classification of Diseases (ICD) categories, and deriving incidence rates from the medical and demographic databases. Rates of hospitalization incidence and prevalence will then be computed per post-deployment person-year for each of the troop types, and these rates will be contrasted to determine if significant differences exist between veterans of the two conflicts.

EXPECTED PRODUCTS (MILESTONES): The study expects to answer the question of how the incidence rates of hospital admissions among GWV compare with the admission rates of veterans deployed to previous combat operation. Similarly, comparisons of the types of medical conditions both groups of veterans were hospitalized for will be determined. Findings should

permit a solid groundwork for policy determinations regarding predeployment interventions in future conflicts and treatment requirements for currently affected troops.

STATUS/RESULTS TO DATE: Data from the Marine Corps unit diaries has been extracted manually and entered into a database. These records include the service numbers of 13,000 Marines deployed to Vietnam at the conclusion of that conflict within Infantry, Support, and Service Support units. Also extracted were any administrative transactions during those last months. The next step is to match these service numbers against a social security number/service number file and obtain the identifiers necessary to pull off service departure dates and medical records from existing demographic and inpatient databases. Parallel Marine units deployed to the Gulf War have been identified and medical and demographic information have already been extracted.

PUBLICATIONS: none to date

Title: Long-term Effects of Subchronic Exposure to Sarin, Alone and with Stress or Other Chemicals

Project #: DoD-72

Agency: DoD

Study Location: Duke Univ. Med. Center

Project Status: Ongoing

Research Type: Mechanistic

P.I.: M. Abou-Donia, Ph.D.

Research Focus: Interactions, Chemical Weapons, Brain & Nervous System

Start Date (CY):

Est. Completion (CY): 2000

OVERALL PROJECT OBJECTIVE: This study will examine, in an animal model, the early and delayed toxic consequences of subclinical sarin exposure given intramuscularly. Additionally, the study will examine the interactive toxicity of the following agents/factors which are considered putative etiological agents for Gulf War Illness: stress, heat, pyridostigmine bromide (PB), DEET, and permethrin. Experiments on blood brain barrier (BBB) permeability included in the study may also quantify a possible mechanism for enhanced interactive toxicity.

SPECIFIC AIMS: The primary hypothesis is that exposure to low levels of sarin, a potent inhibitor of acetylcholinesterase, causes neurologic dysfunctions via: a) down regulation of cholinergic functions due to accumulations of acetylcholine or direct binding of sarin to acetylcholine receptors, and/or b) increased permeability of the blood brain barrier (BBB) resulting in the possible entrance of xenobiotics, and humeral or immunological factors. The goal is, therefore, to assess the possible long-term, delayed toxic effects of low-level, subclinical exposure to the nerve agent sarin, alone, or in combination with other factors.

METHODOLOGY: The study includes the following experiments: 1) dose-finding study for sarin; 2) effect of stress on subclinical sarin exposure; 3) effect of heat on subclinical sarin exposure; 4) effect of PB on subclinical sarin exposure; 5) effect of combined stress and PB on subclinical sarin exposure; 6) effect of combined heat and PB on subclinical sarin exposure, and; 7) effect of combined stress, heat, PB, DEET, and permethrin on subclinical sarin exposure. Neurologic deficits will be assessed by: a) clinical condition, b) neurobehavior, c) integrity of the blood brain barrier, and d) electrophysiological changes.

EXPECTED PRODUCTS (MILESTONES): The proposal should provide mechanistic information to form a basis for determining adverse health effects of environmental conditions associated with the Gulf War and for determining long-term consequences of sarin exposure and the degree of interaction, if any, between stressors, other chemical agents and sarin. A particular strength is the examination of alterations over long time periods following short-term sarin exposure.

STATUS/RESULTS TO DATE: The following studies have been carried out: 1. The intramuscular LD50 dose of Sarin was determined to be 0.1mg/kg in male Sprague-Dawley rats. 2. A dose-response study on the effect of Sarin at 1xLD50, 0.5 x LD50, 0.1xLD50, and 0.01 LD50 on acetylcholinesterase, acetylcholine muscarinic receptors, blood-brain barrier has been carried out

and the results are being analyzed. 3. The effect of chemical exposure has been carried out and the results are being analyzed. 4. The effect of stress has been carried out and the results are being analyzed. 5. Plans are being made for behavioral studies after receiving the equipment needed to carry out these studies. 6. Electrophysiological studies are about to start.

PUBLICATIONS: none to

Title: Post-deployment Morbid Stress, Behavior and Health: Developing a Model for Predicting Morbidity, Mortality, and other Adverse Outcomes

Project #: DoD-73

Agency: DoD

Study Location: Soc. Sectors Develop. Strategies Inc

Project Status: Ongoing

Research Type: Epidemiology Research

P.I.: Nicole Bell, Sc.D, MPH

Research Focus: Symptoms/General Health

Start Date (CY):

Est. Completion (CY): 2001

OVERALL PROJECT OBJECTIVE: The goals of this study are to evaluate the utility of using an existing dataset containing health and administrative records; document and describe morbidity, mortality and other outcomes among soldiers serving in the Persian Gulf; and identify important demographic, behavioral and stress-related factors associated with excess morbidity as determinants of whether or not a soldier will develop a "war syndrome" condition subsequent to deployment or combat. The overall hypothesis is that "Gulf War Syndrome" (GWS) symptomology (health outcomes: as indicated by GWS hospitalizations, Comprehensive Clinical Evaluation Program registration, injuries, and deaths and accidents) result from four main factors and effect modifiers on the first three of these factors. The four factors are: predeployment stressors, distress and functional status (as indicated by information culled from the Defense Manpower Data Center [DMDC] Total Army Injury and Health Outcomes Database [TAIHOD] including Health Risk Appraisals [HRA] filled out by individual troops); predeployment health behaviors (again as indicated by DMDC and HRA); Deployment and related stressors (as indicated by DMDC data); and Post-deployment behaviors (culled from DMDC and HRA data).

SPECIFIC AIMS: Specific Aims of the study are to document and describe morbidity and mortality among army soldiers who are veterans of the Gulf War; identify key demographic, behavioral and stress related factors associated with excess morbidity or mortality among these veterans; document variations in health related behaviors and stress among veterans; and measure associations between these behaviors, stress and health. Nine specific hypotheses will be tested: a) that there is a baseline prevalence of the conditions most commonly included in "Gulf War Syndrome" always present among members of the active duty Army; b) that individual characteristics and experiences of stress explain some variation in presentation of Gulf War Syndrome in the general population independent of deployment to the Gulf War; c) that selection for deployment to the Gulf War is non-random and occurs in such a way that those at greatest risk for development of behavioral or stress related health problems are also those most likely to be deployed; d) that the addition of deployment to the Gulf improves the model describing associations between individual characteristics, stress and subsequent development of Gulf War Syndrome; e) that variations in stressors experienced during the war among deployed troops explain some variations observed among veterans in terms of who does and who does not ultimately present with Gulf War Syndrome; f) that the combination of information including pre-war measures of health and stress, individual characteristics, deployment to the gulf, stressors occurring concurrent with the time spent in the Gulf, can be used to predict much of the variation in Gulf War Syndrome; g) that those who experience the greatest amount of stressors during the war are most likely to adopt risky health behaviors or to report higher levels of distress postwar; h) that a deterioration in health behaviors and/or an increase in stress measures after the war correlate with an increased risk for adverse health outcomes; and i) that effect modifiers of stress.

distress, functional status and health behaviors will improve the model's fit and facilitate understanding of factors key to predicting variation among those who do and do not develop Gulf War Syndrome.

METHODOLOGY: Data from the Total Army Injury and Health Outcomes Database (TAIHOD) will be used for the study analysis. This database includes all hospitalizations, deaths, disabilities and accidents reported for the years 1980-1997. It also includes detailed personnel records containing information about demographic characteristics, occupation, deployments, and discharge information. Construction of variables used in testing each hypothesis will occur after careful consideration of the fields of the DMDC database and after constructs to measure stressors, distress, functional status and job control/demand are developed.

EXPECTED PRODUCTS (MILESTONES): The study may provide a predictive model for health outcomes among soldiers in future conflict situations which may be used in policy decisions regarding who is deployed in future conflicts.

STATUS/RESULTS TO DATE: The project is on track and meeting expected goals as stated in the time line. We have obtained CCEP registry data and matched to our master files. We have updated, error checked, and cleaned our major predictive database, the Health Risk Appraisal. We have developed a widely respected team of expert consultants and have met several times to address the conceptual challenges faced in defining Gulf War Illnesses and to hash out plans for an appropriate analytic plan. We have begun analyses for the first two papers. One will examine temporal trends in hospitalizations for conditions commonly included in Gulf War Illness groupings, the other explores factors which predict variation among those who did and those who did not deploy to the Persian Gulf including prior health status and personal risk factors like risk taking behaviors and experience of stress and distress. We expect to have manuscripts prepared within a month.

PUBLICATIONS: none to

Title: Relationship of Stress Exposures to Health in Gulf War Veterans

Project #: DoD-74

Agency: DoD

Study Location: Duke Univ. Med Center

Project Status: Ongoing

Research Type: Epidemiology Research

P.I.: John Fairbank, Ph.D.

Research Focus: Symptoms/General Health

Start Date (CY):

Est. Completion (CY): 2002

OVERALL PROJECT OBJECTIVE: Research on Gulf War illnesses (GWI), leaves many questions unanswered about diagnostic syndromes of GWI, dimensions of stress and stress exposures encountered by Gulf War (GW) veterans, relationships of stressors and GW health syndromes, and factors that may mediate relationships. The proposed study has six key aims that will begin to address these gaps and enhance understanding of undefined GWI.

SPECIFIC AIMS: The six key aims of the study are: 1) to identify and examine dimensions of illnesses and health problems commonly reported by GW veterans; 2) to assess exposures to stress and to identify the specific variables comprising stress; 3) to examine the extent to which exposures to particular dimensions of stress during deployment and participation in Operations Desert Shield and Desert Storm (ODS/S) are associated with the commonly reported and undefined post-war health problems of GW veterans; 4) to clarify how premilitary and predeployment adversities, risk factors, and protective factors affect GWI outcomes; 5) to examine the role of perceived exposure to environmental toxins during ODS/S; and 6) to examine the mediating role of post ODS/S factors (e.g. instrumental and emotional social support, general psychological functioning, specific co-morbid psychiatric disorders: e.g. post-traumatic stress disorder [PTSD] and depression), and GWI outcomes.

METHODOLOGY: These aims will be achieved by obtaining comprehensive probability based

data from national samples of GW veterans and non-GW veterans provided by files maintained by the Defense Manpower Data Center. Initially research will focus on obtaining information on background factors, health conditions, psychological functioning, and illnesses through a large-scale mail survey stratified by gender (men, women), military component (active, reserve) and GW deployment experience (deployed, not deployed to GW theater). These data will be used to identify dimensions of GWI and to classify respondents into groups with and without GWI and/or related symptoms. A subsample of deployed respondents with and without symptoms of GWI will then be stratified by military component and gender and selected to participate in a computer assisted telephone interview to obtain in-depth information about military stress exposures, psychological disorders, co-morbid or supportive conditions, premilitary and predeployment adversities, risk factors, and protective factors. Multivariate models will be developed for men and women and will compare outcomes for respondents with and without GWI.

EXPECTED PRODUCTS (MILESTONES): Findings from the study will have high significance for understanding the role of a wide range of risk and protective factors on the health of GW veterans and for identifying key explanatory variables that will begin to unravel these complex relationships. More specifically, the study will identify dimensions of GWI and stressors, the relationships among them, and mediating effects of risk and protective factors. Evidence of multiple dimensions of nonspecific health problems (aim 1) may suggest that there are subtypes of GWI that could have treatment implications for veterans suffering from GWI. Gaining a clearer understanding of factors and mechanisms that underlie dimensions of deployment and/or war stressors (aim2) has implications for ways to bolster current intervention and prevention efforts, such as those implemented by combat stress control detachments. Findings on risk and protective factors for chronic, nonspecific health problems have implications for improving military preparedness for future deployments and conflicts (aims 3 to 6).

STATUS/RESULTS TO DATE: Months 1-4 of the project have focused on finalizing the sampling design and measures.

PUBLICATIONS: none to

Title: Toxic Interactions of Prophylactic Drugs and Pesticides

Project #: DoD-75

Agency: DoD

Study Location: Duke Univ

Project Status: Ongoing

Research Type: Mechanistic

P.I.: M. Abou-Donia, Ph.D.

Research Focus: Interactions, Pyridostigmine Bromide, Brain & Nervous System

Start Date (CY):

Est. Completion (CY): 2003

OVERALL PROJECT OBJECTIVE: The goal of this project is to clarify interactive effects and mechanisms of combined exposure to three compounds: pyridostigmine bromide (PB), a prophylactic treatment for nerve gas; the insect repellent DEET; and the insecticide permethrin.

SPECIFIC AIMS: The studies in this project will expand on findings that combined exposure to combinations of these chemicals produce greater toxicity than that produced by single components by testing exposures to doses low enough to be representative of human exposure levels during the Gulf War. The hypothesis is that exposure to a mixture of chemicals reduces the body's ability for their detoxification and results in increased concentration of the compounds in the circulation and enhanced delivery to the vulnerable organ sites with a resultant toxicity.

METHODOLOGY: These studies will be carried out by administering test compounds in the rats, for 60 days as follows: 1) the mechanisms of drug-pesticide interactions will be investigated by conducting dose-response studies using a range of doses including real life doses of human exposure levels plus 10- and 100 times these doses. A tiered approach using single compounds as well as binary and tertiary mixtures will be studies. Animals will undergo behavioral evaluation and at termination. brain. blood. and liver esterases will be assayed as well as liver cytochrome

p450; 2) the integrity of the blood brain barrier will be evaluated by determining: a) the permeability to [3H]hexamethonium iodide, b) acetylcholinesterase activity, and c) immunohistochemical studies, d) physiologically based pharmacokinetic modeling of single compounds and mixtures.

EXPECTED PRODUCTS (MILESTONES): Results of the project should provide mechanisms of interactive effects of combined exposure to the anti-nerve gas agent prophylaxis, pyridostigmine bromide (PB); the insect repellent, DEET; and the insecticide, permethrin.

STATUS/RESULTS TO DATE: We are currently getting ready to start the studies of this project that has just been awarded.

PUBLICATIONS: none to date

Title: Evaluations of Immunotoxicity due to Concurrent Exposure to DEET, Pyridostigmine, and JP-8 Jet Fuel

Project #: DoD-76

Agency: DoD

Study Location: Med Univ of SC, Charleston

Project Status: Ongoing **Research Type:** Mechanistic

P.I.: Deborah Keil, Ph.D.

Research Focus: Interactions, Pyridostigmine Bromide, Immune Function

Start Date (CY):

Est. Completion (CY): 2002

OVERALL PROJECT OBJECTIVE: This project elaborates on information from recent studies which indicates that neurotoxicity increases during concurrent exposure to both pyridostigmine bromide (PYR) and N,N-diethyl-m-toluamide (DEET) with other agents. Based on known interdependencies of the immune and nervous system, the investigators propose that exposure to PYR and DEET will negatively impact the immune system. Additionally, as independent, low-level exposures of JP-8 jet fuel significantly affects T and B lymphocyte populations, the investigators propose to determine the consequences of low-level interactions of JP-8 with DEET and PYR on immune protection mechanisms and manifestations of disease conditions affected by immune mediation.

SPECIFIC AIMS: The hypothesis of this study is that manifestations of Gulf War Syndrome which include adenopathy, fever, joint pain, and skin rashes are linked to immune dysfunction due to exposure to agents such as DEET, PYR, and JP-8 jet fuel. The aim of this project is to provide a comprehensive immunological evaluation to examine the potential effects of subchronic, low-level, concurrent exposures of DEET, PYR, and JP-8.

METHODOLOGY: The study will determine the effects of these three common military agents on specific immunological parameters (i.e. natural killer cell activity, macrophage function, etc.) and susceptibility to diseases (e.g. cancer, infections, and autoimmune disease).

EXPECTED PRODUCTS (MILESTONES): Results of the project studies will provide information on the effects of subject compounds, administered singly or in combination, on the specified aspects of the immune system.

STATUS/RESULTS TO DATE: none reported.

PUBLICATIONS: none to date

Title: Percutaneous Absorption of Chemical Mixtures Relevant to the Gulf War

Project #: DoD-77

Agency: DoD

Study Location: CCTRP, NC State Univ., Raleigh

Project Status: Ongoing

Research Type: Mechanistic

P.I.: Jim E. Riviere. Ph.D.

Research Focus: Interactions, Pyridostigmine Bromide

Start Date (CY):

Est. Completion (CY): 2002

OVERALL PROJECT OBJECTIVE: The purpose of this project is to quantitate the dermal absorption And cutaneous toxicity of chemical mixtures that veterans may have been exposed to during the Persian Gulf War. Veterans are thought to have been exposed to chemical mixtures containing: an insect repellent (DEET); an insecticide (permethrin); the prophylactic drug pyridostigmine bromide, PB; perhaps chemical warfare agents, and jet fuel. The study will evaluate the effects of chemical mixtures and chemical-biological interactions to which Gulf War veterans may have been exposed.

SPECIFIC AIMS: See objectives.

METHODOLOGY: Full and partial factorial design experiments will be used to evaluate potential chemical-chemical and chemical-biological interactions when these chemical mixtures are topically applied to porcine skin. Porcine skin, which is structurally and physiologically similar to human skin, will be utilized in these in vitro and ex vivo studies. The first series of experiments will involve flow-through diffusion experiments to evaluate the dermal absorption of mixtures. The next series of experiments will determine whether isolated perfused porcine skin flaps (IPPSF) affected by a systemic toxin (PB), alters the dermal absorption of topically applied chemicals. The studies will also determine the most sensitive biomarkers (prostaglandin release, morphological changes) of dermatological effects associated with these mixtures. The final series of experiments will evaluate absorption of DEET and permethrin in similarly PB perfused IPPSFs that were topically treated with permethrin.

EXPECTED PRODUCTS (MILESTONES): These studies will experimentally determine which chemical mixtures significantly influenced the absorption of toxicants relevant to the Gulf War Illness.

STATUS/RESULTS TO DATE: This grant is projected to be awarded February 1, 1999; and, thus, research has not yet been initiated.

PUBLICATIONS: Baynes RA, Halling KB, Riviere JE: The influence of diethyl-m-toluamide (DEET) on percutaneous absorption of permethrin and carbaryl. Toxicology and Applied Pharmacology, 144:332-339, 1997.

Baynes RA, Monteiro-Riviere NA, Qiao GL, Riviere JE: Cutaneous toxicity of the benzidine dye Direct Red 28 applied as a mechanistically-defined chemical mixture (MDCM) in perfused porcine skin. Toxicology Letters, 93:159-169, 1997

Title: Experimental Models of Gulf War Syndrome

Project #: DoD-78

Agency: DoD

Study Location: NY Univ Med Center

Project Status: Ongoing

Research Type: Mechanistic

P.I.: Hugh Evans, Ph.D.

Research Focus: Interactions, Chemical Weapons, Brain & Nervous System

Start Date (CY):

Est. Completion (CY): 2002

OVERALL PROJECT OBJECTIVE: This project will develop experimental models with laboratory Animals which can be used to identify possible causes and therapies for the "Gulf War Syndrome" which includes persistent problems that are dominated by cognitive, neurological and respiratory complaints. Unique features of the proposal are: these are the first experiments with animals receiving dynamic inhalation exposures to OP; documentation of early effects using near-real time assays and bio-electronic sensors to identify fundamental physiological mechanisms which can be extrapolated to humans: and the study of combined exposures to OP, PB, and

Stress. Because visualization as to whether the toxic effects are reversible is essential to risk assessment, the long-term (up to 180 days post-exposure) consequences will be established.

SPECIFIC AIMS: See objectives.

METHODOLOGY: In order to clarify the causal factors, rats and macaques will be evaluated before and after low level exposures to the following suspected causative agents: inhalation of the organophosphate (OP) compounds: Soman (SO) or Sarin (SA); oral exposure to the anti-cholinesterase medication pyridostigmine (PB); and psychological stress resulting from exposure to novel stimuli (Stress). Chemical exposures will be quantified in terms of the concentration of the chemical in the atmosphere and by biomarkers of exposure in blood. Toxic effects will be measured by well-established end points that can be used with experimental animals and later with humans. The end-points used are cognitive function (continuous performance, pre-pulse inhibition), diurnal rhythms in locomotion, sensory evoked potential, and pulmonary function (lung capacity, airway conductance and responsiveness).

EXPECTED PRODUCTS (MILESTONES): Quantitative models will be established to allow more focused and mechanistically driven human studies in the future and to allow development of better organophosphate (OP) antidotes from future animal model studies. Chemical exposures will be quantified in terms of the concentration of the chemical in the atmosphere. Specific biomarkers of exposure effects will be produced which can be used in future experiments with animals and humans. And, consequences of long term exposure to the chemicals will be determined to aid in risk assessment.

STATUS/RESULTS TO DATE:

PUBLICATIONS: none to date

Title: Time Course of Stress-induced Impairment of Blood Brain Barrier

Project #: DoD-79

Agency: DoD

Study Location: WRAIR, Wash. DC

Project Status: Ongoing

Research Type: Mechanistic

P.I.: G. J. Kant, Ph.D.

Research Focus: Pyridostigmine Bromide, Brain & Nervous System

Start Date (CY):

Est. Completion (CY): 2000

OVERALL PROJECT OBJECTIVE: Determine whether sustained stress impairs the blood brain barrier, thus permitting brain entry to drugs that normally do not cross the blood brain barrier. Specifically determine whether the chemical defense pretreatment drug pyridostigmine which normally does not cross the blood brain barrier gains access to brain under conditions of sustained stress. This issue is relevant to soldiers serving in the Gulf War who received pyridostigmine. In addition, determine whether pyridostigmine might interact with stress so as to exacerbate the effect of stress on physiology and behavior.

SPECIFIC AIMS: Measure levels of pyridostigmine in the brains of control and chronically stressed rats that are administered pyridostigmine. Measure levels of cholinesterase, the enzyme affected by pyridostigmine, in the brain and blood of control and stressed rats administered pyridostigmine. Measure stress hormones and cognitive performance in control and stressed rats receiving vehicle or pyridostigmine.

METHODOLOGY: To model sustained stress such as soldiers might experience, utilize a rat chronic stress paradigm well characterized in our laboratory. Rats live 24 hr/day in operant cages with intermittent signaled presentation of footshock which is avoidable and escapable by one rat of each pair which pulls a chain to avoid or escape shock for itself and its partner. Rats avoid or escape > 99% of shock presentations. This stress exposure results in elevated levels of plasma stress hormones, decreased food intake and weight gain, decreased thymus weight, disrupted biological rhythms, disrupted sleep patterning, impaired performance on cognitive tasks, similar to the effects of stress in humans. Control and stressed rats will be implanted with indwelling

osmotic pumps that deliver pyridostigmine or vehicle and then exposed to the stress exposure.

EXPECTED PRODUCTS (MILESTONES): We feel that our will provide paradigm a useful model for Soldier operational stress and can be used to elucidate the physiological and behavioral sequelae of stress exposure.

STATUS/RESULTS TO DATE:

PUBLICATIONS: none to date

Title: Molecular Regulation of Corticosteroid Receptor Expression in Stress-Responsive Cells

Project #: DoD-80

Agency: DoD

Study Location: USUHS, Bethesda MD

Project Status: Ongoing

Research Type: Mechanistic

P.I.: Jeff Harmon, Ph.D.

Research Focus: Brain & Nervous System

Start Date (CY):

Est. Completion (CY): 2001

OVERALL PROJECT OBJECTIVE: The purpose of this investigation is to elucidate the mechanisms responsible for the regulation of glucocorticoid receptor (GR) expression in tissues of the HPA axis. The hypothalamic-adrenal-pituitary (HPA) axis is an essential component of the body's response to acute stress, providing a tightly controlled negative feedback loop to regulate the synthesis and release of the adrenal stress hormone cortisol. However, in experimental models of chronic stress, the normal circadian rhythm of corticosteroid release is perturbed, and HPA axis response to corticosteroid challenge becomes abnormal. In addition, abnormal HPA axis response is often seen in major depression, and in patients suffering from posttraumatic stress disorder (PTSD), suggesting that at least some clinical manifestations of these disorders can be attributed to aberrant HPA axis function. Although the basis for abnormal HPA axis response is unknown, experimental models suggest that abnormalities of GR expression can result in aberrant HPA axis function and may contribute to some cognitive and endocrinological deficits seen in major depression.

SPECIFIC AIMS: The overall goal of this research is to elucidate the role of the HPA axis in homeostatic stress response and how abnormal HPA axis function can contribute to stress-related illnesses such as major depression and PTSD. The specific goals are to: 1) determine the extent of alternative human GR promoter utilization, and identify and map each promoter; 2) characterize the cis-acting regulatory elements associated with each GR promoter; and 3) identify tissue specific trans-acting factors responsible for differential (positive or negative) GR autoregulation.

METHODOLOGY: To achieve these objectives, each human GR 5'-end will be used to identify its corresponding genomic sequence. For 5'-sequences not contained within available genomic clones, human chromosome libraries will be screened to isolate the relevant genomic clones, which will then be mapped and sequenced. The functional activity of each putative promoter will be evaluated by construction of chimeric reporter genes and transfection into a panel of cell lines representing various stress-responsive tissues (pituitary, hypothalamus, adrenal) relevant to regulation of the HPA axis. To identify important cis-acting regulatory elements, transient transfection assays conducted in an appropriate panel of cell lines will be used to evaluate the effects of corticosteroids, adrenocorticotropin (ACTH), and corticotropin releasing hormone (CRH) on the activity of each promoter. Mutational analysis of cis-acting elements known to regulate other genes will be used to determine if these elements also regulate GR expression. In addition, deletion analysis will be used to identify other cis-acting elements. Elements identified in these experiments will be evaluated (alone and in combination) for activity in heterologous systems using both minimal and complex promoters to define potential interactions between elements. Tissue-specific trans-acting factors will be characterized in two ways. Nuclear extracts prepared from appropriate cell lines will be assayed for the ability to alter the electrophoretic mobility of the

cis-acting elements we identify. The identity of factors interacting with known elements will be determined/confirmed by competition experiments with nonspecific and mutated oligonucleotides, and "supershift" experiments with appropriate antibodies. Where the identity of a trans-acting factor cannot be ascertained, it will be purified and partial peptide sequence data obtained. In addition, the activity (as measured by electrophoretic mobility shift, and/or immunoreactivity) will be measured in appropriate cells and tissues after treatment with corticosteroids, ACTH, and CRF. Successful completion of this work will provide important new information about the regulation human GR gene expression, and thus the regulation of the HPA axis. Only with such an understanding can the abnormal function of the HPA axis in a variety of stress-related disorders be understood, and appropriate interventions developed to restore normal function.

EXPECTED PRODUCTS (MILESTONES): Isolation and characterization of functional promotor(s); identification of cis-acting elements; identification of trans-acting transcription factors.

STATUS/RESULTS TO DATE: Funding is still pending.

PUBLICATIONS: Liu W, Hillman AG, Harmon JM. Hormone-independent repression of AP-1 inducible collagenase promoter activity by glucocorticoid receptors. *Mol Cell Biol*, 15:1005-1013, 1995.

Sivo J, Harmon JM, Vogel SN. Heat shock mimics glucocorticoid effects on IFN-induced FcRI and Ia mRNA expression in mouse peritoneal macrophages. *J Immunol*, 156:3450-3454, 1996.

Ramdas J, Harmon JM. Glucocorticoid-induced apoptosis and regulation of NF-B activity in human leukemic T cells. *Endocrinology*, 139:3813-3821, 1998.

Ramdas J, Liu W, Harmon JM. glucocorticoid-induced cell death requires autoinduction of glucocorticoid receptor expression in human leukemic T cells. (submitted)

Title: Immunotoxicity due to Coexposure to DEET, Pyridostigmine, and Stress

Project #: DoD-81

Agency: DoD

Study Location: VAMC Charleston

Project Status: Ongoing

Research Type: Mechanistic

P.I.: Gary Gilkeson, M.D.

Research Focus: Interactions, Immune Function, Pyridostigmine Bromide

Start Date (CY):

Est. Completion (CY): 2001

OVERALL PROJECT OBJECTIVE: Gulf War soldiers were exposed concurrently to agents such as N,N-diethyl-m-toluamide (DEET) and pyridostigmine bromide (PYR), however, it is not clear whether coadministration of these agents results in significant health effects or contributes to the signs and symptoms of "Gulf War Syndrome" reported by the Gulf War veterans. Likewise, it is not known whether elevated levels of physical stress may also influence potential toxicity during coexposure to DEET and PYR. Independent exposures of PYR or DEET are reported to cause minimal physiological effects in humans. However, recent studies indicate increased neurotoxicity during multi-chemical exposures including PYR and/or DEET with or without environmental stress. The effect of combined exposure on the immune system is unknown; it is possible these agents alone or in combination have no direct effect on immune function. The immune system, however, directly interacts with the nervous system in a variety of ways. Thus, if there were direct effects of these agents on the nervous system, they may also have indirect effects on immune function. Consequently, it is rational to elucidate potential effects on immune function and susceptibility to cancer, infectious, and autoimmune disease due to PYR and DEET coadministered in the presence of stress.

SPECIFIC AIMS: 1) To test the effect of subchronic coexposures to low-levels of DEET. PYR.

and physiological stress on immunotoxicological, hypersensitivity, and inflammatory responses in B6C3F1 mice. 2) To assess the effect of concurrent exposure to DEET, PYR, and physiological stress on susceptibility to infectious disease and/or cancer in B6C3F1 female mice. 3) To determine if subchronic concurrent exposure to PYR, DEET, and physiological stress will accelerate autoimmune disease in autoimmune prone MRL-lpr/lpr mice.

METHODOLOGY: Using mice as a model for the human immune system, we hypothesize that subchronic coexposure to low-levels of DEET, PYR, and physiological stress will significantly affect immunological function and increase susceptibility to cancer, infectious disease or autoimmune disease.

EXPECTED PRODUCTS (MILESTONES): Ultimately, these studies may provide insight into the mechanisms of disease for the manifestations experienced by Gulf War veterans.

STATUS/RESULTS TO DATE: We are just beginning the initial studies doing dose-ranging studies of the individual agents.

PUBLICATIONS: none to date

Title: Feasibility of Developing a Registry of PTSD Affected Veteran Sib Pairs

Project #: DoD-82

Agency: DoD

Study Location: VAMC St. Louis

Project Status: Ongoing

Research Type: Epidemiology Research

P.I.: Seth Eisen, M.D.

Research Focus: Brain & Nervous System

Start Date (CY):

Est. Completion (CY): 2000

OVERALL PROJECT OBJECTIVE: 1) From a cohort of possible sib pairs in which both sibs are likely to have PTSD, randomly sample 2,000 sib pairs (4,000 individuals) to determine: a. the proportion of true sib pairs, and b. the proportion of sib pairs who can successfully be contacted; 2) From the true sib pair cohort of possible sib pairs at risk for PTSD, determine the proportion who are screened PTSD positive by telephone interview; 3) From the PTSD screened positive sib pairs defined in #2, determine the proportion who will provide blood for a linkage study protocol.

SPECIFIC AIMS: The proposed feasibility study will determine whether Department of Defense (DoD) and Department of Veterans Affairs (VA) computer based files can be linked to identify sib pairs who have had PTSD at some time in their lifetime.

METHODOLOGY: The Defense Manpower Data Center (DMDC) of the DoD will analyze their database of over 11 million veterans to identify possible sib pairs using algorithms which match veterans for same last name, different first name, and similar social security number or address at induction into military service. A PTSD enriched data base of veterans who have been diagnosed or treated by the VA for PTSD, will be merged with the DMDC database of probable sib pairs to give a database of probable sib pairs in which one or both members are at high risk for PTSD. Military records located at the National Personnel Records Center in St. Louis will be utilized to confirm sib pair status. A random sample of confirmed sib pairs in which one or both members are at high risk for having PTSD will be screened via telephone interview to identify PTSD affected sib pairs. A subset of this group will be invited to contribute a blood sample. Cooperation rates for these steps will be carefully analyzed.

EXPECTED PRODUCTS (MILESTONES): This "affected sib pair" registry can provide the basis to begin to investigate genetic loci associated with PTSD. If we can demonstrate that it is feasible to develop a PTSD affected sib pair registry of sufficient size, and that registry members are willing to be interviewed and provide a blood sample, then a large scale linkage study will be proposed.

STATUS/RESULTS TO DATE: Considerable pilot work has been completed. In cooperation with DMDC, a database of approximately 150,000 possible sib pairs has been developed. In addition, a database of 235,000 veterans who have been diagnosed or treated by the VA for PTSD has

been developed, and these two data sets have been merged. We are now preparing to review records at the NPRC to confirm sib status, and abstract military service data that is associated with increased risk for having PTSD (e.g., service in Vietnam, awarded combat related medals). The project is successfully adhering to its original timetable.

PUBLICATIONS: none to date

Title: Risk for Stress-related Substance Abuse: the Effects of Family History of Alcoholism

Project #: DoD-83

Agency: DoD

Study Location: USUHS, Bethesda, MD

Project Status: Ongoing

Research Type: Clinical Research

P.I.: Frances Gabbay, Ph.D.

Research Focus: Brain & Nervous System, Prevention

Start Date (CY):

Est. Completion (CY): 2001

OVERALL PROJECT OBJECTIVE: Individuals with a family history of alcoholism are at increased risk for alcohol and other substance abuse disorders. Evidence from twin and adoption studies suggests strongly that this increase in risk is due to genetic as well as environmental factors. In a multifactorially-determined disorder with some genetic contributions, biological relatives of affected persons may be carrying components of the biological predisposition without being affected themselves. The family-history method identifies subjects who are at high risk for developing alcoholism by virtue of their family history, but who are themselves not alcoholic (family-history positive, FHP). These individuals are matched and compared to nonalcoholic offspring of control subjects with no family history of alcoholism (family-history negative, FHN), permitting analyses of factors that predispose individuals to alcohol and other substance abuse without the confounding effects of heavy alcohol or other drug use. This design has resulted in a body of research that has begun to reveal differences in attentional processes between FHP and FHN subjects. There is preliminary evidence that these apparent differences in the allocation of attention, inferred from differences in the P300 component of the event-related brain potential (ERP), may be mined to distinctive patterns of arousal in the two groups.

SPECIFIC AIMS: We will employ the family-history design to test hypotheses derived from the assumed differences in arousal: (1) in tasks requiring sustained attention, FHP subjects will fatigue more quickly than FHN subjects; (2) FHP subjects will show greater response to d-amphetamine than FHN subjects in that d-amphetamine will counteract fatigue related changes to a greater extent in FHP subjects; and (3) stimulus novelty will have a greater effect in reversing fatigue related changes in FHP subjects than in FHN subjects. Our tests of these hypotheses will use sensitive electrophysiological measures of attention and arousal in conjunction with measures of task performance and measures of subjective effects. In addition, we will test the hypothesis that (4) the effects on our measures of a family history of alcoholism will be less evident in women than in men.

METHODOLOGY: We will recruit a sample of FHP (n=30) and FHN (n=30) adults, comprising equal numbers of women and men, using state-of-the-art methods to define and assess family history of alcohol and other substance abuse. A between-subjects design will be used to study the effect of family history on our measures. A within-subject design will be employed to permit examination of the effects of fatigue, d-amphetamine dose, and stimulus novelty. Testing will be double-blind, and will consist of three sessions. Over the course of these three sessions, subjects will receive a placebo, 10 mg, and 15mg of d-amphetamine, packaged in matching capsules. The test battery will comprise the following: (1) visual and auditory versions of the Identical Pairs Continuous Performance Test (IP-CPT), a demanding test of sustained attention that is very sensitive to fatigue; (2) an auditory novelty oddball task, which permits evaluation of processes underlying the detection and processing of stimuli outside the focus of attention; (3) the resting EEG, a classic measure of arousal; and (4) self-report of subjective effects, using the Profile of

Mood States (POMS), the Visual Analog Scale (VAS), and the Biphasic Alcohol Effects Scale (BAES). The test battery will be administered three times in each session: once before, and 30 and 180 minutes following capsule ingestion. The novelty oddball task will be included only in one test battery, the one that begins 180 minutes following drug ingestion. Family-history group differences in the effects of d-amphetamine dose and stimulus novelty on fatigue-related changes in our electrophysiological and behavioral measures will be assessed. Sex differences in the effects of our variables will also be examined.

EXPECTED PRODUCTS (MILESTONES): Substance abuse is evident in the military as well as the civilian population. Moreover, it has been reported that stress related to military assignments may contribute to substance abuse by military women and men. Thus, the relevance of this proposal to the VA/DoD mission arises from its focus on the use of behavioral and electrophysiological measures to differentiate individuals at varying risk for substance abuse, as well as from its aim to address the question of whether--and if so, how--the effects of a family history of alcoholism are different for women and men.

STATUS/RESULTS TO DATE: The project was to have begun in Oct. 1998, but due to difficulties with the grant funding, it has not yet begun.

PUBLICATIONS: none to date

Title: Psychobiologic Alterations in Persian Gulf War Veterans with and without PTSD

Project #: DoD-84

Agency: DoD

Study Location: VAMC Bronx

Project Status: Ongoing

Research Type: Clinical Research

P.I.: Julie Golier, Ph.D.

Research Focus: Brain & Nervous System, Symptoms/General Health

Start Date (CY):

Est. Completion (CY): 2002

OVERALL PROJECT OBJECTIVE: Many of the veterans who served in the Gulf War theatre have subsequently developed somatic and psychological symptoms. In some veterans the symptoms are clearly due to known disorders. For example, it has been estimated that 8-12% of Gulf War veterans (GWVs) are suffering from Posttraumatic Stress Disorder (PTSD). Many others are experiencing a broad array of symptoms which defy diagnostic classification. It has been hypothesized that the unexplained symptoms are due to war zone stress, but empirical evidence is lacking. To evaluate whether symptoms in GWVs are related to a stress disorder, we propose to study psychobiologic alterations in GWVs using the same neuroendocrinologic methods that have proven useful in elucidating the pathophysiology of PTSD in Vietnam veterans. Dr. Yehuda, one of the co-investigators, has previously identified a distinct profile of neuroendocrine alterations which is associated with PTSD. Importantly this profile is distinct from the typical biological response to chronic stress and distinct from the alterations seen in other psychiatric disorders, such as depression.

SPECIFIC AIMS: Using these same neuroendocrine methods, we aim to determine whether PTSD is associated with neuroendocrine alterations in GWVs. Similarly, we aim to evaluate whether other health symptoms in these veterans are associated with neuroendocrine alterations. To do so, we will characterize the veterans according to the factor-analytically derived "Gulf War Syndrome", factors identified by Dr. Robert Haley. These factors are 'impaired cognition', 'confusion-ataxia', and 'arthro-myo-neuropathy'.

METHODOLOGY: To the extent that neuroendocrine alterations similar to those previously observed in classic PTSD are present in symptomatic GWVs, or subsets of these veterans, it might be appropriate to consider treating these veterans using current strategies for the treatment of PTSD. To the extent that the pattern observed in symptomatic GWVs is unlike the pattern typical of PTSD, this would suggest that some other pathophysiology underlies these symptoms and that different treatment strategies may be needed. Additionally, the identification of patterns

of neuroendocrine alterations could aid in the biological subtyping of Gulf War health symptoms. The specific neuroendocrine tests we propose to use are: the low-dose dexamethasone suppression test (DST), the metyrapone stimulation test (MST), lymphocyte glucocorticoid receptor (GR) activity, and circadian analysis of cortisol, ACTH, norepinephrine (NE) and 3-methoxy-5-hydroxy phenol glycol (MHPG). We will examine whether there are differences between GWVs grouped by PTSD diagnosis on these neuroendocrine measures. Additionally, we will assess the relationship between Gulf War Syndrome factors and these same biological measures, as well as the relationship between the Gulf War Syndrome factors and PTSD.

EXPECTED PRODUCTS (MILESTONES): By using the same neuroendocrine battery in GWVs with PTSD as we have previously studied in Vietnam veterans, and are currently studying in veterans of Korea and WWII, we will also be in a unique position to compare the findings among groups of combat veterans. If different groups of combat veterans with PTSD show the same neuroendocrine profile, it will suggest that the alterations are an essential feature of PTSD. If they differ, we will be able to explore whether the biologic alterations of PTSD vary according to other factors such as age, magnitude of combat exposure, or course of illness.

STATUS/RESULTS TO DATE: none reported.

PUBLICATIONS: none to date

Title: CNS Cytokines and CRH in Gulf War Veterans with Multiple Unexplained Symptoms

Project #: DoD-85

Agency: DoD

Study Location: VAMC Cincinnati

Project Status: Ongoing

Research Type: Clinical Research

P.I.: Dewleen Baker, M.D.

Research Focus: Brain & Nervous System, Treatment, Symptoms/General Health

Start Date (CY):

Est. Completion (CY): 2002

OVERALL PROJECT OBJECTIVE: To longitudinally determine CSF IL-6, IL-1 and CRH as well as plasma ACTH and cortisol concentrations in GWVs with and without unexplained physical symptoms and in a comparison fibromyalgia control group. Veterans in each of the three study groups will be studied using serial CSF sampling techniques.

SPECIFIC AIMS: see objectives.

METHODOLOGY: We propose to use the technique of continuous CSF sampling to determine the relationship between multiple physical symptoms and concentrations of CNS cytokines, as well as to assess the function of the HPA axis in GWVs. Specifically, we will test the following hypotheses: (1) GWVs with multiple unexplained symptoms will have significantly higher CSF and/or peripheral IL-6 levels and IL-1-beta concentrations than those veterans without physical complaints. (2) CSF CRH concentrations in GWVs with multiple physical symptoms will be low in comparison with the CSF CRH concentrations in asymptomatic GWVs but CSF CRH concentrations will be similar to those of a fibromyalgia control group. Thirty seven serial measurements of cytokines and neuroactive substances will be obtained at 10 minute intervals, beginning 3 h after flexible subarachnoid catheter insertion. We have helped pioneer the serial CSF sampling technique to be used and find that it is extremely well-tolerated and safe and has major scientific advantages over lumbar puncture. Blood will be withdrawn at 10 minute intervals during CSF sampling to harvest plasma for serial cortisol and ACTH concentrations. Additionally, 24-h urinary free cortisol (UFC) levels will be obtained.

EXPECTED PRODUCTS (MILESTONES): Veterans mobilized in the Persian Gulf War were exposed to an especially wide range of stressors known to persistently activate inflammatory cascades including combat, smoke from oil fires, organophosphates and pyrostigmine prophylaxis, immunizations and possible chemical or biological warfare agents. Symptoms of GWVs - fatigue, headache, musculoskeletal pains and memory loss - resemble those of cytokine mediated "sickness behaviors" and of stress-related conditions. such as fibromyalgia. Central

nervous system (CNS) abnormalities of cytokines and their neurohormone modulators, which to our knowledge have yet to be evaluated in GWVs, could provide an explanation for the unexplained symptoms and a target for pharmacotherapy development.

STATUS/RESULTS TO DATE: none reported.

PUBLICATIONS: Baker DG, Boat B, Grinvalsky HT, Geraciotti TD. Interpersonal and Animal Related Trauma in Female and Male Military Veterans, *Military Medicine*, 163:20-25, 1998.
Geraciotti, TD, Loosen PT, Ekhtator NN, Schmidt D, Chambliss B, Baker DG, Kaskow JW, Richtand NM, Keck PE, Ebert MH. Uncoupling of Serotonergic and Noradrenergic Systems in Depression: Preliminary Evidence from Continuous Cerebrospinal Fluid Sampling, *Depression* 6:89-94, 1997.

Kellner M, Baker DG, Yehuda R. Salivary Cortisol in Operation Desert Storm Returnees, *Biological Psychiatry*, 42:849-850, 1997.

Geraciotti TD, Goldsmith JR, Friedman LM, Norman AB, Somoza E, Kasckow JW, Baker DG, Richtand NM, Anthenelli RM, Keck PE. Cerebrospinal Fluid Neuroendocrinology of Alcohol Misusers, *Addiction Biology* 2:401-409, 1997.

Baker DG, Mendenhall CL, Simbarti LA, Megan LK, Steinberg JL. Relationship between Posttraumatic Stress Disorder and Self-Reported Physical Symptoms in Persian Gulf War Veterans, *Archives of Medicine*, 157:2076-2078, 1997.

Baker DG, West SA, Orth DN, Hill KK, Nicholson WE, Ekhtator NN, Bruce AB, Wortman MD, Keck PE, Geraciotti TD. Cerebrospinal Fluid and Plasma-Endorphin in Combat Veterans with Posttraumatic Stress Disorder. *Psychoneuroendocrinology*, 22:517-529, 1997.

Title: Effects of Combat Stress on Structure and Function of the Hippocampus

Project #: DoD-86

Agency: DoD

Study Location: VAMCs Boston & Palo Alto

Project Status: Ongoing

Research Type: Clinical Research

P.I.: Dan Kaloupek, Ph.D.

Research Focus: Brain & Nervous System

Start Date (CY):

Est. Completion (CY): 2001

OVERALL PROJECT OBJECTIVE: Sapolsky and colleagues demonstrated widespread cell death in selected regions of the hippocampus in primates subjected to severe crowding stress. This atrophy appears to be mediated by the glucocorticoid receptors located in the affected regions. Recently, magnetic resonance (MR) imaging has suggested similar vulnerability to stress in the human hippocampus. Studies by Bremner and colleagues (1995,1997), Gurvitz et al. (1996), and Stein et al. (1997) have all demonstrated reduced hippocampal volume in target high-stress groups (e.g., Vietnam veterans with combat-related PTSD) compared to control groups. Despite scientific limitations, the apparent consistency of the findings has generated excitement in the field and impetus for careful replication.

SPECIFIC AIMS: The primary aim is to replicate the seminal studies on reduced hippocampal volume in PTSD patients with more sensitive measurement methods and with design elements that address the potentially confounding influence of chronic alcohol abuse. The fore-most secondary aim is to reconcile inconsistencies between the observations of hippocampal volume reduction and two independent lines of evidence that are broadly incompatible with the concept of glucocorticoid-mediated hippocampal atrophy. An additional aim is to address questions of immediate medical significance to the VA and DoD, specifically the potential amplification of hippocampal aging by stress and the role, if any, of the hippocampus in the severe memory complaints of some Persian Gulf War (PGW) veterans.

METHODOLOGY: There are six study groups, three involving combat exposed Vietnam veterans

(males only) and three involving Persian Gulf-deployed veterans (males and females). Subjects are recruited from PTSD and Environmental Medicine clinics in VA, from the general community, and from contacts made through a centralized database for PGW veterans. The Vietnam cohort includes two groups with current PTSD, one with no history of alcohol abuse and one with such a history. It also includes a group with no current mental disorder and no history of alcohol abuse. The PGW cohort includes comparable PTSD and No-Disorder groups, both having no history of alcohol abuse, plus a third group characterized by self-identified complaints of memory dysfunction in the absence of either current PTSD or lifetime alcohol abuse. Within each cohort, subjects with PTSD are compared to subjects without PTSD. In addition, within the Vietnam cohort, subjects with PTSD and a history of alcohol abuse/dependence are compared to subjects with PTSD who do not have a history of alcohol abuse/dependence. Within the PGW cohort, subjects who report severe memory problems in the absence of PTSD are compared to subjects without memory problems or diagnosed mental disorder. A cross-cohort analysis compares groups of Vietnam and POW veterans with and without PTSD, all of whom are without a history of alcohol abuse. Finally, gender differences are tested in an exploratory fashion in the POW groups.

EXPECTED PRODUCTS (MILESTONES): The primary aim is addressed by comparing groups of traumatized Vietnam veterans with and without histories of alcohol abuse, by increasing the sensitivity and reliability of volumetric estimations through enhanced-resolution MR imaging (Wald et al, 1995), and by adding more comprehensive assessments of memory. The main secondary aim is addressed by obtaining measures of circulating glucocorticoids (via salivary cortisol) and memory-related processing (via event related potentials) on the same individuals for whom hippocampal volume is determined. This multimethod approach brings together indices of anatomical structure, HPA activation, electro-physiological processing, memory performance, and stress, and provides a unique opportunity for the integration of findings across domains of measurement and function. The medical significance of the findings is addressed by examining the wide-ranging measures for indications of accelerated hippocampal aging in Vietnam veterans with PTSD and for correlates of severe memory complaints in the PGW population.

STATUS/RESULTS TO DATE: Research support staff are being recruited, and other operational preparations are being made to initiate data collection.

PUBLICATIONS: Kounios J, Litz BT, Kaloupek DG, Riggs D, Knight J, Weathers FW, Anderson J, Keane TM. Electrophysiology of combat-related PTSD. In R. Yehuda & A.C. McFarlane (Eds.), *Psychobiology of Post Traumatic Stress Disorder* (pp. 504-507). New York: Annals of the New York Academy of Sciences, 1997.

Orr SP, Kaloupek DG. Psychophysiological assessment of PTSD. In J.P. Wilson & T.M. Keane (Eds.) *Assessing Psychological Trauma and PTSD* (pp. 69-97). New York: Guilford, 1996.

Woodward SH, Friedman MJ, Bliwise DL. Sleep and depression in combat-related PTSD inpatients. *Biological Psychiatry*, 39, 182-192, 1996

Title: Measurement and Validation of Psychosocial Risk and Resilience Factors Accounting for Physical and Mental Health and Health-Related Quality of Life among PGWVs

Project #: DoD-87

Agency: DoD

Study Location: VAMC Boston

Project Status: Ongoing

Research Type: Epidemiology Research

P.I.: Daniel King, Ph.D.

Research Focus: Brain & Nervous System, Prevention

Start Date (CY):

Est. Completion (CY): 2001

OVERALL PROJECT OBJECTIVE: This project has the long-term objective of producing a reliable inventory of psychosocial risk and resilience factors for contemporary military personnel and then demonstrating its validity vis-a-vis Persian Gulf War veterans' self-reported somatic and psychological symptoms and judgments of health-related quality of life. The inventory will include assessments of six dimensions of war-zone stress (exposure to combat, exposure to the grotesque aftermath of battle, anticipatory fear or perceived threat, lower magnitude aspects of the malevolent war-zone environment, concern about life and family disruption, and sexual harassment); two predeployment vulnerabilities (prior trauma history and quality of family functioning); and two reentry postwar circumstances (social support and additional stressful life events).

The overriding working hypothesis for the proposed project is that scores on the psychosocial risk and resilience inventory will be meaningfully related to self-reported physical and mental health and health-related quality of life, thus validating the inventory itself. This global proposition will find support in the testing of six subsidiary hypotheses. The first three hypotheses are aimed at documenting main effects of the psychosocial risk and resilience predictors on outcomes, controlling for self-reported exposures to chemical or biological agents. Two hypotheses are intended to delineate expected interactions between veteran demographic characteristics and the psychosocial risk and resilience factors, controlling for self-reported exposures to chemical or biological agents. The sixth hypothesis postulates possible synergistic consequences of self-reported exposures to chemical or biological agents and the presence of one or more psychosocial risk and resilience factor(s).

SPECIFIC AIMS: The project has three specific objectives, each corresponding to a separate phase: (1) To define and operationalize the variables representing psychosocial risk and resilience that are proposed to influence veterans' self-reported health outcomes; (2) To collect data from a test development sample of Persian Gulf War veterans and conduct first-stage psychometric analyses; and (3) To relate scores on the refined war-zone, predeployment, and reentry-postwar measures to indices of physical and mental health and health-related quality of life. This third specific objective subsumes the evaluation of a series of hypotheses using data from a second-stage test validation sample of Persian Gulf War veterans.

METHODOLOGY: The project will adhere to the scientifically-based process of construct validation as a guiding framework to generate a conceptually meaningful and psychometrically sound inventory. As with virtually all instrument development projects, the research design is observational and cross-sectional. Two stratified random samples of Persian Gulf War veterans, a test development sample and then a test validation sample, will be drawn with the assistance of personnel from the Defense Manpower Data Center. Data will be collected by telephone interviews conducted by Schuman, Ronca, and Bucuvalas, Inc. (SRBI), a well-regarded market and opinion survey research firm. Initial data analysis will generate classical test theory-oriented item and scale characteristics and estimates of internal consistency reliability. The testing of all hypotheses will be conducted using hierarchical multiple regression analyses.

EXPECTED PRODUCTS (MILESTONES): Findings are intended to provide information to assist military leaders to better prepare personnel for future deployments and DoD and VA health-care policy-makers and practitioners to plan and implement more effective prevention and treatment programs. The major product, a portable risk and resilience assessment device, will be a standard tool for use by other researchers (psychosocial, biomedical, or otherwise) and a prototype measure of psychosocial features of future deployments.

STATUS/RESULTS TO DATE: Hiring of staff has been partially accomplished, and initial arrangements for focus groups with PGWVs have been made.

PUBLICATIONS: none to date

Title: Clinical Relevance of Novel Immunological Markers in PTSD

Project #: DoD-88

Agency: DoD

Study Location: VAMC White River Junction

Project Status: Ongoing

Research Type: Clinical Research

P.I.: Peter Morganelli, Ph.D.

Research Focus: Immune Function, Brain & Nervous System

Start Date (CY):

Est. Completion (CY): 2002

OVERALL PROJECT OBJECTIVE: The purpose of this proposal is to study the expression of a novel glucocorticoid-regulated glycoprotein specific to human monocytes (the p155 antigen) in PTSD. The overall goal is to determine whether levels of expression of p155 or other immune system parameters, or changes in levels of expression in a dexamethasone suppression test are associated with symptoms and severity of PTSD. In vitro, expression of p155 is regulated by physiologically relevant amounts of free glucocorticoids, where a half-maximal enhancement of expression occurs with concentrations of hormone that half-saturate the glucocorticoid receptor. Thus, baseline expression of p155 on peripheral monocytes, and/or enhancement of expression in response to DEX administration in vitro may represent a sensitive index of hypothalamic-pituitary-adrenal (HPA) axis activity in PTSD. We propose a case-control comparison of current PTSD versus current major depressive disorder versus nonpsychiatric controls.

SPECIFIC AIMS: See objectives.

METHODOLOGY: For these studies, we will recruit and screen male Vietnam war veterans in VA health care and from the community to assemble the following study groups: (a) 15 subjects with a current PTSD diagnosis, (b) 15 subjects with a current depression diagnosis but no lifetime PTSD, and (c) 15 subjects with no lifetime PTSD or depression diagnosis and no current Axis I psychiatric disorder (controls). PTSD diagnosis will be confirmed or ruled out by the Clinician Administered PTSD Scale. Major depression and other psychiatric diagnoses will be confirmed or ruled out by the Structured Clinical Interview for DSM-IV. Also, the severity of PTSD and depressive symptomatology will be assessed by validated questionnaires. In these subjects, we will measure (a) monocyte expression of p155, (b) levels of the cytokines IL-1 beta and IL-10 in serum, (c) their production by mononuclear cells in vitro, and (d) total plasma cortisol. Baseline p155 expression, and expression after treatment with glucocorticoids in vitro will be measured with monoclonal antibodies by quantitative flow cytometry. In addition, p155 expression and plasma cortisol will also be measured in each subject after administration of the synthetic glucocorticoid dexamethasone (DST). Data will be analyzed in relation to plasma cortisol levels and in relation to PTSD diagnostic and severity status.

EXPECTED PRODUCTS (MILESTONES): The data obtained from these studies will not only provide new information of the relationship between HPA axis activity and immune function in PTSD, but may also allow the development of a simple and sensitive test useful in the diagnosis and management of PTSD.

STATUS/RESULTS TO DATE: none reported.

PUBLICATIONS: none to date

Title: Limbic Blood Flow and Opiate Receptor PET in Posttraumatic Stress Disorder

Project #: DoD-89

Agency: DoD

Study Location: VAMC Ann Arbor

Project Status: Ongoing

Research Type: Clinical Research

P.I.: Lorraine Fig, M.D.

Research Focus: Brain & Nervous System, Symptoms/General Health

Start Date (CY):

Est. Completion (CY): 2002

OVERALL PROJECT OBJECTIVE: Using positron emission tomography (PET) we propose to characterize the amygdaloid complex function in PTSD. We hypothesize that abnormal amygdaloid complex activation is involved in the pathophysiology of PTSD and in PTSD symptom

generation, and that a likely neurochemical basis for this abnormality is alteration in the density of opioid receptors within the amygdala.

SPECIFIC AIMS: Specifically, we hypothesize that: 1) Greater regional cerebral blood flow (rCBF) activation, in the amygdaloid complex and associated limbic regions of PTSD patients, will be demonstrated in response to both traumatic and non-specific aversive emotional stimuli, relative to control groups. 2) Increased mu-opioid receptor binding will be demonstrated in the amygdaloid complex and associated limbic regions of PTSD patients relative to control groups. 3) The magnitude of rCBF activation in the amygdaloid complex and associated limbic regions will positively correlate with mu-opioid receptor binding in the same region.

METHODOLOGY: Three groups of 20 subjects each will be studied: 1) Male Vietnam veterans with PTSD; 2) Non-combat normal controls, matched by age and gender; 3) Combat controls: Vietnam War veterans, matched by age and gender, who served in active combat in the Vietnam theater, but do not have a history of PTSD or meet criteria for active disease. Subjects with organic mental disorder or psychoactive substance dependence will be excluded. The psychiatric diagnosis will be established using SCID IV for patients and SCID NP for controls. Each group undergoes two types of PET studies: 1) Characterization of the baseline mu-opioid receptor availability and regional distribution using [C-11] Carfentanil (a selective mu-opioid receptor agonist) in PTSD patients and control groups. 2) Using [O-15] water regional cerebral blood flow measurements and an activation paradigm developed and validated in our laboratory, we will examine the limbic system and amygdaloid complex responses to trauma-specific, non-specific negative and neutral emotional stimuli (viewing emotionally negative versus neutral pictures and listening to trauma-specific versus neutral scripts) in PTSD patients and control groups.

EXPECTED PRODUCTS (MILESTONES): The proposed study offers a novel approach that simultaneously examines both neuroanatomical and neurochemical hypotheses of PTSD, linking seemingly isolated findings into an integrated view of PTSD pathophysiology. This combined approach may elucidate both the functional neuroanatomy of symptom generation and the underlying neurochemical processes that "drive" this function. The within-subject design permits examination of the strength of association between the changes in blood flow and neurochemical findings, providing valuable information on rCBF regulation in the limbic system in general. PTSD specific findings would establish a background for our understanding of the brain mechanisms underlying PTSD and, ultimately, new intervention strategies based on a rational neurochemical hypothesis. Finally it will also provide a new research tool for evaluation of treatment outcomes for PTSD which currently afflicts at least 470,000 Vietnam veterans and has a major health care delivery and financial impact on the VHS and DOD.

STATUS/RESULTS TO DATE: none reported.

PUBLICATIONS: Environmental Exposure Report, Depleted Uranium in the Gulf:

<http://www.gulflink.osd.mil/du> Environmental Exposure Report, Oil Well Fires:

<http://www.gulflink.osd.mil/envexp.html> Draft Environmental Surveillance Health Risk Assessment,

Kuwait Oil Fires: <http://www.gulflink.osd.mil/envexp.html>

Title: SPECT Benzodiazepine Receptor and MR Imaging in PTSD

Project #: DoD-90

Agency: DoD

Study Location: VAMC West Haven

Project Status: Ongoing

Research Type: Clinical Research

P.I.: Robert Innis, M.D.

Research Focus: Brain & Nervous System, Treatment

Start Date (CY):

Est. Completion (CY): 2001

OVERALL PROJECT OBJECTIVE: The overall goals of this application are to use state-of-the-art neuroimaging methodologies to measure a receptor (BZ/GABA_A) which is believed to play a critical role in anxiety and to measure the hippocampus (which is involved with memory function)

in patients with PTSD. We will study Gulf War and Vietnam War veterans with PTSD in comparison to control groups. This study will measure potential underlying neurochemical and neuroanatomic substrates associated with the disorder and will help to assess the time frame required for such abnormalities to develop, since the Gulf War was more recent than the Vietnam War.

SPECIFIC AIMS: A large number of preclinical studies demonstrate an important role for GABA_A receptors in generating symptoms of anxiety and of being down-regulated by repeated stress. Furthermore, the GABA_A receptor is known to mediate the anxiolytic effects of benzodiazepines. We have found a significant decrease in BZ receptor binding in left hippocampus and precuneus in patients with panic disorder, which shares many symptoms with PTSD.

METHODOLOGY: Some of the core symptoms of PTSD involve memory disturbances, including intrusive memories of the traumatic event and nightmares. Furthermore, we have found Vietnam veterans with PTSD have deficits on formal memory testing (Bremner et al., 1993a). Since animal studies have shown that stress and high cortisol levels are toxic to the hippocampus, we measured the volume of the hippocampus using MRI. Vietnam veterans with PTSD had reduced hippocampal volume, which may reflect atrophy secondary to the trauma or the recurrent PTSD disorder (Bremner et al., 1995b).

EXPECTED PRODUCTS (MILESTONES): We predict that, in comparison to veterans without PTSD, patients with PTSD will have decreased hippocampal volume, decreased hippocampal and frontal cortical BZ/GABA_A receptor density, and impaired memory function. To assess these possibilities, we will study four groups of subjects: Gulf War veterans meeting DSM-IV criteria for PTSD; National Guard reservists without PTSD and who did not serve in the Gulf War; Vietnam veterans with PTSD; and Vietnam era veterans without PTSD and who did not serve in Vietnam.

STATUS/RESULTS TO DATE: none reported.

PUBLICATIONS: none to date

Title: Neurological and Circadian Substrates of PTSD-like Behaviors

Project #: DoD-91

Agency: DoD

Study Location: VAMC San Antonio/Armstrong Lab

Project Status: Ongoing

Research Type: Mechanistic

P.I.: Alan Frazer, Ph.D.

Research Focus: Brain & Nervous System

Start Date (CY): Est. Completion (CY): 2001

OVERALL PROJECT OBJECTIVE: We hypothesize that the genetic susceptibility to PTSD lies in those brain systems and brain regions where the processes of behavioral plasticity (sensitization) and adaptation to stress converge. A great deal of work implicates central catecholaminergic and serotonergic neuronal systems in the response to stress, and these transmitters are active in anatomic sites known to be linked to the signs and symptoms of anxiety and fear-associated trauma (e.g., amygdala, hippocampus, locus coeruleus) and circadian rhythm generation (i.e., the suprachiasmatic nuclei). Little work has been directed, though, at the mechanisms underlying genetic susceptibility for a maladaptive stress response. This should be possible using strains of animals that exhibit abnormal behavioral and physiological responses to stress. Such animals are valuable tools to study the link between disease states and stress. Our intention is to use a strain of rat vulnerable to stress effects, namely the Wistar Kyoto (WKY) strain, to address this problem. Also, there has been very little work on PTSD-relevant processes such as sensitization in such strains. We will study such phenomena using amygdala kindling as a priming stimulus leading to sensitization.

SPECIFIC AIMS: We hypothesize that the sensitizing process of amygdala kindling will induce region-specific differences in the brain noradrenergic and/or serotonergic systems of WKY as compared to Wistar (W) rats, and that differences in the response of these biogenic amine systems to acute stress will be most obvious following sensitization, reflecting the genetic

vulnerability of the WKY rat. Finally, we hypothesize that kindling will cause changes in circadian function (amplitude, photic sensitivity) and fear potentiated startle, and that these changes will be more pronounced in the WKY strain.

METHODOLOGY: This overall hypothesis will be tested in a collaborative project involving investigators from the VA and the DOD. In particular, investigators at the VA will determine if: 1) in the absence of acute stress, there are region-specific differences in parameters reflective of noradrenergic or serotonergic function in brains of WKY versus W rats; 2) an acute stress (restraint) causes different changes in brain noradrenergic or serotonergic function in WKY compared to W rats; 3) amygdaloid kindling cause changes in the neurobiologic parameters measured, and if larger effects are produced in WKY than in W rats; 4) kindling differentially alters the physiologic and neuronal responses of WKY and W rats to acute restraint stress.

Measurement will be made of plasma catecholamines, ACTH, and corticosterone in response to a 30 min restraint stress. mRNA for tyrosine hydroxylase in the adrenal medulla will be measured and various indices of noradrenergic and serotonergic function will be evaluated in specific brain areas by in situ hybridization histochemistry and quantitative autoradiography. These measurements will be carried out both prior to and after the restraint stress, and in rats either exposed to or not exposed to amygdala kindling.

Investigators at the DOD will use WKY and W rats to: 1) determine internal phase relationships among overt rhythms; 2) characterize the response of the circadian system to light; and 3) characterize circadian rhythm in fear-potentiated startle. This will be done in rats both prior to and after amygdala kindling.

EXPECTED PRODUCTS (MILESTONES): The results of these studies should provide important information linking stress with disease states in vulnerable individuals. This relatively novel approach could illuminate the nature of the genetic/environment interaction that is necessary for the development of PTSD.

STATUS/RESULTS TO DATE: none reported.

PUBLICATIONS: none to date

Title: Traumatic Experiences Persistently Enhance Cue-dependent Learning: Toward an Animal Model of Chronic Stress and Posttraumatic Stress Disorder

Project #: DoD-92

Agency: DoD

Study Location: VAMC E. Orange

Project Status: Ongoing

Research Type: Mechanistic

P.I.: Richard Servatius, Ph.D.

Research Focus: Brain & Nervous System

Start Date (CY):

Est. Completion (CY): 2002

OVERALL PROJECT OBJECTIVE: Patients with posttraumatic stress disorder (PTSD) exhibit a constellation of signs and symptoms which suggest long term alterations in associative and nonassociative learning (e.g., intrusive imagery, exaggerated startle responses to stimuli reminiscent of the traumatic experience, anniversary reactions). To advance our understanding of how exposure to traumatic experiences translated into these diverse learning-related signs, we have developed an animal model which displays persistent stress-induced alterations in associative and nonassociative learning.

SPECIFIC AIMS: Rats exposed to inescapable stressors display startle sensitization (nonassociative learning) and facilitated acquisition of the classically conditioned eyeblink response (associative learning). These neurobehavioral effects are apparent from 14 days after stressor cessation.

METHODOLOGY: The present proposal will test two hypotheses. First, we will determine whether persistent neurobehavioral abnormalities are dependent on persistent neuroendocrine changes. Next, we will determine whether persistent changes in associative learning influences

the appearance of startle sensitization.

EXPECTED PRODUCTS (MILESTONES): These studies will advance our understanding of how exposure to stressors persistently alter basic learning processes, the development of chronic stress state, and ultimately may provide insight into the development and maintenance of stress-related mental illness, such as PTSD.

STATUS/RESULTS TO DATE: none reported.

PUBLICATIONS: none to date

Title: Troops Exposed to New Agents at Aberdeen Proving Ground - Follow Up Investigation

Project #: DoD-93

Agency: DoD

Study Location: MFUA/IOM

Project Status: Pending

P.I.: Bill Page, Ph.D.

Start Date (CY): 7/1/99

Est. Completion (CY): 2000

OVERALL PROJECT OBJECTIVE: Between 1955 and 1975, the U.S. Army enrolled 6,720 soldiers in an experimental exposure program of chemical warfare and other agents at the Edgewood Arsenal, Maryland. In 1980 the Army asked the National Research Council (NRC) to study the possible long term health effects of these exposures. A three-volume report was issued, the last volume dealing with the current health status of test subjects, including 1,581 men exposed to anticholinesterase compounds such as GA (tabun), GB (sarin), GD (soman), GF, and VX. The report indicated that "the limited information available from the follow-up on these soldiers does not permit definitive conclusions regarding the nature and extent of possible long-term problems resulting from chemical exposure at Edgewood." A pilot study was undertaken to determine whether follow-up of these test subjects is feasible and whether it would provide useful information (see projects VA/DoD-2DA/2VA.)

Taking the suggestion of the NRC committee, we propose to survey by telephone the Edgewood subjects who were exposed to anticholinesterase agents for the more common OP exposure-associated outcomes; neurological deficits, particularly peripheral nerve disease, and neuropsychological impairment, including sleep disorders, anxiety, and depression.

SPECIFIC AIMS:

METHODOLOGY: The Edgewood subjects who were unexposed to chemical agents will serve as the first control group. However, this is not an ideal control group because the original Edgewood protocol did not include random assignment of subjects to treatment and control groups. In particular, it is thought that healthier men were more likely to have been assigned to chemical exposure groups and less healthy men to the control group. To counteract the effect of this putative assignment bias, a second control group consisting of men who were exposed to chemical agents other than anticholinesterases will also be included in the follow-up.

We are proposing a screen by telephone questionnaire of all exposed subjects and controls for neurological deficit and neuropsychological impairment, including sleep disorders, anxiety, and depression.

EXPECTED PRODUCTS (MILESTONES):

STATUS/RESULTS TO DATE: MFUA has discussed the results of this pilot study with the Neurology Dept and the Washington DC VA Medical Center and the Minneapolis MN VA Medical Center. They have agreed to work together if a full-scale study is funded. MFUA will also consult with the staff of the Board on Environmental Studies and Toxicology, National Research Council, with regard to toxicological matters.

PUBLICATIONS: none to date

Title: Health Assessment of Persian Gulf War Veterans from Iowa

Project #: HHS-1

Agency: HHS

Study Location: CDC and state of IA

Project Status: Ongoing

Research Type: Epidemiology Research

P.I.: David Schwartz, MD, MPH

Research Focus: Symptoms/General Health

Start Date (CY): 1994

Est. Completion (CY): 2000

OVERALL PROJECT OBJECTIVE: To assess the prevalence of self-reported symptoms and illnesses among military personnel deployed during the Persian Gulf War and to compare the prevalence of these conditions with the prevalence among military personnel on active duty at the same time, but not deployed to the Persian Gulf.

SPECIFIC AIMS: This study addressed the following specific hypotheses: (1) Persian Gulf War (PGW) veterans experienced more self-reported health problems in the post-war period than did Persian Gulf-era military personnel who did not serve in the Persian Gulf region; (2) active duty military personnel with service in the Persian Gulf region experienced more self-reported health problems in the post war period than did active duty military personnel who did not serve in the Persian Gulf region; (3) National Guard and reserve personnel with service in the Persian Gulf region experienced more self-reported health problems in the post-war period than did National Guard and reserve personnel who did not serve in the Persian Gulf region; and (4) National Guard and reserve personnel with service in the Persian Gulf region experienced more self-reported health problems than active duty military personnel with comparable service.

METHODOLOGY: Data on self-reported health outcomes was collected through a telephone survey with a random sample of Persian Gulf War veterans and Persian Gulf War-era controls who listed Iowa as their home of record. Interviews were completed with 3,695 eligible study subjects (76%).

EXPECTED PRODUCTS (MILESTONES): Initial report published in JAMA on January 15, 1997.

STATUS/RESULTS TO DATE: Initial results indicate that compared with non-PGW military personnel, PGW veterans report a significantly higher prevalence of symptoms of depression, PTSD, chronic fatigue, cognitive dysfunction, bronchitis, asthma, fibromyalgia, alcohol abuse, anxiety, sexual discomfort, and health related quality of life. Additional data analysis is ongoing. A protocol was developed for collecting physical exam data on a subset of the telephone survey participants to validate the self-report of asthma. The asthma validation component is ongoing and expected to be completed by April 2000.

PUBLICATIONS: Iowa Persian Gulf Study Group. Self-reported illness and health status among Gulf War veterans: a population-based study. JAMA, 277:238-245, 1997.

Title: Disease Cluster in a Pennsylvania Air National Guard Unit, EPI-AID 95-18

Project #: HHS-2

Agency: HHS

Study Location: CDC and states of PA & FL

Project Status: Ongoing

Research Type: Epidemiology Research

P.I.: William C. Reeves, MD

Research Focus: Symptoms/General Health

Start Date (CY): 1994

Est. Completion (CY): 2000 Diagnosis

OVERALL PROJECT OBJECTIVE: At request of VA, DoD, Pennsylvania Health Department, CDC investigators conducted an EPI-AID to evaluate reported cluster of illness in the Pennsylvania 193rd Air National Guard.

SPECIFIC AIMS: Characterize patients, determine if there was a cluster of illness, determine if the illness was unique to the 193rd ANG, related to residence in Pennsylvania, or related to PGW service, characterize the illness and derive a working case definition, and identify risk factors for illness.

METHODOLOGY: Stage 1 case series; stage 2 cross sectional population-based interview; stage 3 physical exam, psychometric testing, clinical laboratory, research laboratory assessment and nested case control study.

STATUS/RESULTS TO DATE: The cross-sectional questionnaire survey included 3723 currently active volunteers, irrespective of health status or GW participation, from 4 air force populations. The cross-sectional clinical evaluation included 158 GW veterans from one unit, irrespective of health status. This study involved development of a symptom-based case definition, assessment of case prevalence rate for GW veterans and nondeployed personnel, and analysis of clinical and laboratory findings among veterans who met the case definition. We defined a case as having 1 or more chronic symptoms from at least 2 of 3 categories (fatigue, mood-cognition, and musculoskeletal). The prevalence of mild-to-moderate and severe cases was 39% and 6%, respectively, among 1155 GW veterans compared with 14% and 0.7% among 2520 nondeployed personnel. Illness was not associated with time or place of deployment or with duties during the war. Fifty-nine clinically evaluated GW veterans (37%) were noncases, 86 (54%) mild-to-moderate cases, and 13 (8%) severe cases. Although no physical examination, laboratory, or serologic findings identified cases, veterans who met the case definition had significantly diminished functioning and well-being. Among currently active members of 4 Air Force populations, a chronic multisymptom condition was significantly associated with deployment to the GW. The condition was not associated with specific GW exposures and also affected nondeployed personnel.

PUBLICATIONS: Morbidity and Mortality Weekly Report: Unexplained Illness Among Persian Gulf War Veterans in an Air National Guard Unit, Preliminary Report, August 1990-March 1995. MMWR, 44(23):443-447, 1995.

Fakuda K, Nisenbaum R, Stewart G, et al. Chronic multisymptom illness affecting Air Force veterans of the Gulf War. JAMA 280:981-988, 1998.

Title: Biomarkers of Susceptibility and Polycyclic Aromatic Hydrocarbon (PAH) Exposure in Urine and blood Cell DNA from U.S. Army Soldiers Exposed to Kuwaiti Oil Well Fires

Project #: HHS-3

Agency: HHS

Study Location: NIH/NCI/DCE/LCTP/IVP

Project Status: Complete

Research Type: Mechanistic

P.I.: Miriam Poirier, Ph.D.

Research Focus: Environmental Toxicology, Prevention

Start Date (CY): 1992

Est. Completion (CY): 1998

OVERALL PROJECT OBJECTIVE: To compare PAH level in soil and air with biomarkers of exposure (urinary metabolites), biologically-effective dose (DNA adducts) and individual susceptibility (genetic polymorphisms) for this group of 62 soldiers. This project is technically not a research project. It was an analysis requested by the U.S. Army Environmental Hygiene Agency, now known as the Center for Health Promotion and Preventive Medicine, as a part of their ongoing exposure assessments.

SPECIFIC AIMS: To determine the extent of exposure and the correlation between these various biomarkers. **METHODOLOGY:** Soldiers participating in a USAEHA surveillance project in which ambient PAH monitoring and biomonitoring were performed. Samples of blood and urine were taken in Germany in June (1991). In August, eight weeks after deployment to Kuwait, and in

October after the return to Germany. PAH-DNA adducts were measured by benzo[a]pyrene-DNA dissociation-enhanced lanthanide fluoroimmunoassay (DELFI) and aromatic DNA adducts were measured by 32P-postlabeling in blood cell DNA. Urinary benzo[a]pyrene metabolites have been determined by immunoaffinity chromatography and fluorescence. Glutathione-s-transferase and cytochrome P450 1A1 polymorphisms have been determined by PCR amplification of the DNA.

EXPECTED PRODUCTS (MILESTONES): The anticipated findings were higher pollution in Kuwait accompanied by higher levels of DNA adducts and urinary metabolites.

STATUS/RESULTS TO DATE: By both methodologies, blood cell DNA adduct measurements were lower in Kuwait than in Germany in June and October. The increase in adduct levels in October (compared to August in Kuwait) was statistically significant. Levels of urinary 1-hydroxypyrene-glucuronide (--OH-PG) were also lower in Kuwait than in Germany but the differences were not statistically significant. No differences in biomarker levels were associated with any metabolic polymorphism. The data suggest that the location of the soldiers' duty stations in Kuwait represented a cleaner working environment than Germany. Actual PAH measurements of air and soil in Kuwait showed very low levels of contamination. Literature values for ambient PAH measurements for Germany at the same time are much higher.

PUBLICATIONS: Poirier MC, Weston A, Schoket B, et al. Biomonitoring of U.S. Army soldiers serving in Kuwait in 1991. *Cancer Epidemiology Biomarkers and Prevention*, 7:545-551, 1998.

Title: Suspected Increase of Birth Defects and Health Problems Among Children Born to Persian Gulf War Veterans In Mississippi

Project #: HHS-4

Agency: HHS

Study Location: CDC, Jackson, MS

Project Status: Complete

Research Type: Epidemiology Research

P.I.: Alan Penman, MBChB, MSc, FRCS

Research Focus: Reproductive Health

Start Date (CY): 1994

Est. Completion (CY): 1996

OVERALL PROJECT OBJECTIVE: To determine the nature and extent of a reported increase of birth defects and health problems among children born to Persian Gulf War veterans from two units of the Mississippi National Guard.

SPECIFIC AIMS: To calculate the number and rate of birth defects and health problems in children born to Persian Gulf War veterans from two units of the MS National Guard after their return from overseas service, and determine whether an excess of birth defects/health problems occurred.

METHODOLOGY: Descriptive survey by medical record review, supplemented by parent/physician interview as required. Comparison of observed numbers/rates with expected numbers/rates from birth defect surveillance systems, epidemiological studies, etc.

EXPECTED PRODUCTS (MILESTONES): Final report published by CDC on 12/19/94 (Field Epidemiology Report #95-01).

STATUS/RESULTS TO DATE: The total number of all types of birth defects was not greater than expected. The frequency of premature birth, low birth weight, and other health problems appeared similar to that in the general population.

PUBLICATIONS: Penman AD, Tarver RS. No evidence of increase in birth defects and health problems among children born to Persian Gulf War veterans in Mississippi. *Military Medicine*, 161(1)1-5, 1996.

Title: Cognitive Function and Symptom Patterns in Persian Gulf Veterans

Project #: HHS-5

Agency: HHS

Study Location: CDC, Boston Univ Sch Public Health

Project Status: Ongoing

Research Type: Clinical Research

P.I.: David Ozonoff, MD, MPH

Research Focus: Brain & Nervous System, Symptoms/General Health

Start Date (CY): 1997

Est. Completion (CY): 2000

OVERALL PROJECT OBJECTIVE: 1) Delineation of a neuroanatomical/neurophysiological basis, if any, for the reported cognitive dysfunction in some Gulf War veterans; 2) Delineation of symptom patterns shown by some individuals who served in the Gulf War; and 3) verification of research findings in an independent cohort of soldiers who served in the Gulf shortly after cessation of hostilities.

SPECIFIC AIMS: 1) Examine differences in degree of activation present on fMRI scans in specific neuroanatomical regions within the frontal lobe, particularly the cingulate gyrus area and the dorsolateral prefrontal cortex, in Gulf War veterans challenged with a test of working memory; 2) Use a mathematical technique of Logical Analysis of Data (LAD) to analyze the relationships of symptom responses in Gulf-deployed and non-Gulf-deployed subjects; and 3) Replicate symptom prevalence comparisons and neuropsychological tests in a Danish cohort of Gulf-deployed and non-Gulf-deployed soldiers.

METHODOLOGY: 1) Both conventional MRI and functional imaging will be used to assess changes in brain activation, to examine symptom profile, and to examine the relationship between functional activation and clinical state. Subjects will be selected from an ongoing Boston Environmental Hazards Center study of neuropsychological function and will include 40 symptomatic Gulf War-deployed veterans, 40 non-symptomatic Gulf War-deployed veterans, 40 Germany-deployed veterans and 30 treatment-seeking non-Gulf War-deployed veterans. 2) LAD will be used to examine previously collected symptom data from Gulf-deployed and Germany-deployed veterans (n=300) to see if there is a set of complaints characteristic of service in the Gulf region useful for determining etiology or for case definition. 3) Neuropsychological test results and symptom prevalence measures will be replicated and verified in a cohort of Danish armed forces. Neuropsychological function and symptom prevalence of Danish troops deployed to the Gulf (n=200) will be compared with non-deployed Danish troops (n=100).

EXPECTED PRODUCTS (MILESTONES):

STATUS/RESULTS TO DATE: Human subjects approval for the fMRI component of the study was obtained in September 1998; subjects are currently being recruited and tested. The response to initial recruitment letters has been very positive. Analyses of individual subjects' fMRI data are being completed as they complete the testing; comparative group analyses will be completed after all subjects have been tested. Human subjects/OPRR approval for the Danish component of the study was obtained in February 1999; subjects are currently being recruited. Data analysis of health symptoms and neuropsychological test performances for the LAD component of the study is ongoing.

PUBLICATIONS: none to date

Title: Defining Gulf War Illness

Project #: HHS-6

Agency: HHS

Study Location: CDC, Univ of Med & Dent of NJ-Robert Wood Johnson Med School

Project Status: Ongoing

Research Type: Clinical Research

P.I.: Howard Kipen, MD, MPH

Research Focus: Diagnosis, Symptoms/General Health

Start Date (CY): 1997

Est. Completion (CY): 2000

OVERALL PROJECT OBJECTIVE: To characterize and identify alternative classifications for symptoms and functional disability which remain medically unexplained in Gulf War veterans. **SPECIFIC AIMS:** 1) To assess the persistence and stability of symptoms over time, and to compare the performance of data-driven case definitions previously derived from two samples of Gulf War veterans (the New Jersey Center for Environmental Hazards Research (NJCEHR) sample of Department of Veterans Affairs Gulf War Registry participants and a CDC sample of Air Force veterans.) Standard or existing case definitions for unexplained multi-symptom illnesses (such as chronic fatigue syndrome, fibromyalgia, and multiple chemical sensitivity) will be simultaneously evaluated to determine if these definitions or data-driven definitions better characterize Gulf War veterans unexplained illnesses. 2) To assess the generalizability of derived and existing case definitions in a new random sample of deployed and non-deployed Gulf war era veterans and active duty soldiers. 3) To assess the role of psychiatric conditions in Gulf War veterans unexplained illnesses.

METHODOLOGY: All subjects from the NJCEHR cohort (N=1,161) and a sample of the CDC cohort (N=1,200) will be asked to complete a telephone survey assessment of symptoms and medical conditions. The stability of symptoms will be assessed by comparing the previous assessment (Time 1) with the current assessment (Time 2). Case definitions derived using factor analytic procedures with Time 1 data will be compared to case definitions derived from Time 2 data. Generalizability of the case definitions will be determined by asking a new randomly selected national sample of Gulf War veterans and era controls (N=3,000) to complete the telephone survey. Psychiatric conditions will be assessed by including the Brief Symptom Inventory (BSI) and the Short form of Composite International Diagnostic Interview (CIDI-SF) in the telephone survey. In order to validate self-reports of serious organic medical conditions, medical record reviews will be completed on a random sample of 70 veterans from the NJCEHR cohort and 70 matched controls who report no serious medical conditions. Finally, as the original NJCEHR study was conducted using a mail survey, one-third of the NJCEHR sample (N87) will be asked to complete a short mail survey as well as the telephone survey in order to assess potential bias of the method of data collection.

EXPECTED PRODUCTS (MILESTONES):

STATUS/RESULTS TO DATE: The protocol has been finalized and has been submitted for human subjects and OMB approval.

PUBLICATIONS: none to date

Title: Immunotoxicity of Dermal Permethrin and Cis-Urocanic Acid

Project #: HHS-7

Agency: HHS

Study Location: Virginia Polytech Inst

Project Status: Ongoing

Research Type: Mechanistic

P.I.: Steven Holladay, Ph.D.

Research Focus: Immune Function, Interactions, Environmental Toxicology

Start Date (CY):

Est. Completion (CY): 2001

OVERALL PROJECT OBJECTIVE: To examine immunotoxicity resulting from combined dermal exposure to permethrin and sunlight. Providing new data to assist the estimation of risk from the combined immunotoxicant exposure (cUCA and topical insecticide) is a primary goal of the proposal.

SPECIFIC AIMS: Inhibited immune responses have been observed following occupational, inadvertent, or therapeutic exposure to xenobiotics. Further, aberrant immune responses (e.g.,

hypersensitivity, autoimmunity) appear to be increasing in humans, a phenomenon which may be related to environmental chemical exposure. New research initiatives are determining that such exposures, often previously considered to be innocuous, may in fact be contributing to impaired human immune health. The present proposal considers immunotoxicity resulting from combined dermal exposure to a common pyrethroid insecticide (permethrin) and to cis-urocanic acid (cUCA, an isomerization product of trans-UCA and sunlight). Preliminary data have been generated showing both systemic and regional immunotoxicity from low-level topical permethrin (formerly not considered an immunotoxicant). It has previously been demonstrated that cUCA also inhibits skin and immune responses.

Permethrin-impregnated uniforms were worn by U.S. troops during the Gulf war. Preliminary studies conducted on permethrin immunotoxicity used topical applications and exposure regimens to simulate continuous or intermittent exposures experienced by U.S. troops showed immunotoxic effects.

METHODOLOGY: The proposed studies will estimate the risk of immunotoxicity from combined topical permethrin and intradermal cUCA exposure, using National Toxicology Program-approved testing procedures in C57B1/6 inbred mice. These immunotoxicants will also be co-administered at levels determined to inhibit immune responses in mice, to investigate cytokine-dependent mechanisms by which cUCA and/or permethrin may cause suppression of immunity. Further, in that: 1) cUCA has been shown to inhibit antigen presentation by macrophages, 2) new data suggest the epidermal antigen presenting cell (APC, Langerhans cell [LC]) may be a target of cUCA, and 3) permethrin was shown by us to inhibit skin contact hypersensitivity responses, the effect of single and combined exposure to these agents on antigen presentation by LC will be examined as a mechanism related to immunotoxicity.

EXPECTED PRODUCTS (MILESTONES): Project completion estimated 31 July 2001.

STATUS/RESULTS TO DATE: Nothing to report; project recently initiated.

PUBLICATIONS: none to date

Title: Strategy to Identify Non-Additive Response to Chemical Mixtures

Project #: HHS-8

Agency: HHS

Study Location: Lawrence Livermore Nat Lab, CA

Project Status: **Research Type:** Development

P.I.: John S. Vogel, Ph.D.

Research Focus: Interactions, Environmental Toxicology

Start Date (CY):

Est. Completion (CY): 2000

OVERALL PROJECT OBJECTIVE: Low level exposures to organophosphate (OP) esters are suspected to impact the health of agricultural workers, soldiers, and the general population. Current techniques of study require extrapolation of imprecise data from high dose exposures to the low doses of usual exposures. Biochemical markers of analyses are the most promising indicators of response to the effects of multiple chemical exposures at environmentally relevant doses. Accelerator mass spectrometry (AMS) has the sensitivity and precision to quantify attomole levels of tissue doses or biomarkers of isotopically labeled compounds to precisions of <5%. The overall goal is to demonstrate a new strategy for identifying chemical mixtures that have non-additive biochemical response at relevant low exposures in order to eliminate the current strategies that depend on uncertain extrapolation of high dose, single compound data. If the strategy proves useful, more rapid and meaningful screening of chemical mixture exposures can be implemented to provide better health advisories and regulations for the general public and for specific occupations.

SPECIFIC AIMS: AMS has the sensitivity and precision to quantify attomole levels of tissue doses. It will be used to demonstrate a new strategy for identifying chemical mixtures that have non-additive biochemical responses at environmentally relevant (low) exposures.

METHODOLOGY: AMS will quantify the nerve cell membrane binding of an isotope labeled OP

pesticide marker at sub-ppm exposures. Chemical mixtures of other esters, also at environmentally realistic doses, will be concurrently applied to determine if these multiple exposures to different esters cause non-additive effects in the binding of the marker OP. Mice will be exposed to up to four compounds in multiples, and OP binding in nerves will be assayed for up to three days.

EXPECTED PRODUCTS (MILESTONES): Project completion estimated 31 August 2000.

STATUS/RESULTS TO DATE: Nothing to report; project recently initiated.

PUBLICATIONS: none to date

Title: Mortality Follow-up Study of Persian Gulf Veterans, First Update

Project #: VA-1

Agency: VA

Study Location: VAMC Washington DC

Project Status: Ongoing

Research Type: Epidemiology Research

P.I.: Han Kang, Dr. P.H.

Research Focus: Mortality

Start Date (CY): 1996

Est. Completion (CY): 1999

OVERALL PROJECT OBJECTIVE: VA's Environmental Epidemiology Service is undertaking a retrospective follow-up mortality study of Persian Gulf veterans that will compare the overall mortality rates and cause-specific mortality rate of all 697,000 service members who served in the Persian Gulf theater of operations anytime between August 1990 and May 1991 with those of a comparison group of 803,526 service members randomly selected from various subsets of all service members who were in service anytime between August 1990 and May 1991 but who were not deployed to the Persian Gulf theater.

SPECIFIC AIMS: Are there excess deaths among Persian Gulf veterans from various natural and external causes in comparison to their non-Persian Gulf veteran counterparts?

METHODOLOGY: A retrospective cohort mortality study; 697,000 study subjects, 800,000 controls; Cox regression model, SMR analysis. Vital status has been determined using a VA database known as the Beneficiary Identification and Record Locator Subsystem (BIRLS) and a Social Security Administration file for deaths reported through December 1995. Death certificates have been collected from VA regional offices, Federal Records Centers or from state vital statistics offices. Cause of death are coded by a qualified nosologist using the International Classification of Diseases, 9th revision, without the knowledge of whether the decedent served in the Persian Gulf area.

As the first mortality follow-up of Persian Gulf veterans, this study represents an important first step in addressing the health concerns of Persian Gulf veterans. The study results as reflected in mortality rates should provide an early warning for any possible life threatening health conditions as a consequence of Persian Gulf service. The study will be periodically updated to ascertain any long-term consequences of Persian Gulf service on mortality outcomes.

EXPECTED PROEJCTS (MILESTONES): Data collection is complete. Preliminary results were presented at the 125th Annual APHA meeting in November 1997.

STATUS/RESULTS TO DATE: The original study indicates that the overall mortality rate is higher in Persian Gulf veterans compared to non-deployed era veterans. However, when causes due to accident, suicide and homicide are accounted for, there is no difference in mortality rate between the two groups. The updated mortality follow-up through December 1995 continues to show that excess mortality in Gulf veterans are due to external causes. This study will be periodically updated to ascertain any long-term consequence of Persian Gulf service on mortality outcomes. A manuscript is in preparation for publication.

PUBLICATIONS: Kang H. Bullman TA. Mortality among U.S. veterans of the Persian Gulf

war. The New England Journal of Medicine, 1996, 335:1498-1504.

Title: National Health Survey of Persian Gulf Veterans

Project #: VA-2

Agency: VA

Study Location: VAMC Washington DC

Project Status: Ongoing

Research Type: Epidemiology Research

P.I.: Han Kang, Dr. P.H.

Research Focus: Symptoms/General Health

Start Date (CY): 1994

Est. Completion (CY): 2001

OVERALL PROJECT OBJECTIVE: To estimate prevalence of various symptoms and other health outcomes for Persian Gulf War veterans.

SPECIFIC AIMS: To estimate and compare prevalence of various symptoms, medical conditions, and unexplained illnesses in Persian Gulf war veterans and those of non-Persian Gulf veterans.

METHODOLOGY: A population-based health survey. Several studies are currently underway that investigate the incidence, prevalence, and nature of illnesses in Gulf War veterans. The National Health Survey of Persian Gulf Veterans and Their Family Members is conducted in three phases. VA-2A through VA-2C describe the different phases of this program.

EXPECTED PROJECTS (MILESTONES): Publication in a peer-reviewed journal.

STATUS/RESULTS TO DATE: The proposed study protocol and questionnaire were peer-reviewed and approved by an external scientific oversight committee in April 1995. The proposed survey questionnaire was approved by the Office of Management and Budget (OMB) in September 1995. The initial questionnaires were mailed in November 1995 and the first supplemental questionnaires were mailed in January 1996 to 20,426 non-respondents and individuals with new mailing addresses. Phase I of the survey was completed in June 1996. Phases II was completed in October 1998. Phase III is in process.

PUBLICATIONS: none to date

Title: VA National Survey of Persian Gulf Veterans - Phase I

Project #: VA-2A

Agency:

Study Location: VAMC Washington DC

Project Status: Complete

Research Type: Epidemiology Research

P.I.: Han K. Kang, Dr. P.H.

Research Focus: Symptoms/General Health

Start Date (CY): 1995

Est. Completion (CY): 1996

OVERALL PROJECT OBJECTIVE: To estimate prevalence of various symptoms and other health outcomes for Persian Gulf War veterans.

SPECIFIC AIMS: To estimate and compare prevalence of various symptoms, medical conditions, and unexplained illnesses in Persian Gulf war veterans and those of non-Persian Gulf veterans.

METHODOLOGY: A population-based health survey. Several studies are currently underway that investigate the incidence, prevalence, and nature of illnesses in Gulf War veterans. The National Health Survey of Persian Gulf Veterans and Their Family Members is conducted in three phases. In this Phase I, a questionnaire was mailed to each of 30,000 veterans (15,000 Persian Gulf Veterans; 15,000 non-Persian Gulf Veterans). Multiple follow-up mailings were made to increase the response rate. A total of 15,825 veterans responded after three follow-up mailings.

EXPECTED PROJECTS (MILESTONES): Publication in a peer-reviewed journal.

STATUS/RESULTS TO DATE: Phase I of the survey was completed in June 1996. Analysis of Phase I data in combination with Phase II data is in progress.

PUBLICATIONS: none to date

Title: VA National Survey of Persian Gulf Veterans - Phase II

Project #: VA-2B

Agency: VA

Study Location: VAMC Washington DC

Project Status: Ongoing

Research Type: Epidemiology Research

P.I.: Han K. Kang, Dr. P.H.

Research Focus: Symptoms/General Health

Start Date (CY): 1996

Est. Completion (CY): 1999

OVERALL PROJECT OBJECTIVE: To estimate prevalence of various symptoms and other health outcomes for Persian Gulf War veterans.

SPECIFIC AIMS: To estimate and compare prevalence of various symptoms, medical conditions, and unexplained illnesses in Persian Gulf war veterans and those of non-Persian Gulf veterans.

METHODOLOGY: A population-based health survey. Several studies are currently underway that investigate the incidence, prevalence, and nature of illnesses in Gulf War veterans. The National Health Survey of Persian Gulf Veterans and Their Family Members is conducted in three phases. In this Phase II, telephone interviews were attempted on all non-respondents using a CATI questionnaire which includes a question on reasons for refusal. Efforts to obtain the telephone interview were successful in 5,116 Gulf veterans and controls increasing the overall response rate to 70%. Telephone interviews with non-respondents will assist in assessing potential non-respondent bias and will supplement the postal survey data. In addition, during Phase II, selected self-reported data collected by the postal questionnaire will be validated through records review for 2,000 veterans from each group. Phase II data collection was completed in October 1998.

EXPECTED PROJECTS (MILESTONES): Publication in a peer-reviewed journal.

STATUS/RESULTS TO DATE: The combined Phase I and Phase II data analysis is in process.

PUBLICATIONS: none to date

Title: VA National Survey of Persian Gulf Veterans - Phase III

Project #: VA-2C

Agency: VA

Study Location: VAMC Washington DC

Project Status: Ongoing

Research Type: Epidemiology Research

P.I.: Han K. Kang, Dr. P.H.

Research Focus: Symptoms/General Health, Brain & Nervous System, Diagnosis

Start Date (CY): 1998

Est. Completion (CY): 2001

OVERALL PROJECT OBJECTIVE: To estimate prevalence of various symptoms and other health outcomes for Persian Gulf War veterans.

SPECIFIC AIMS: To estimate and compare prevalence of various symptoms, medical conditions, and unexplained illnesses in Persian Gulf war veterans and those of non-Persian Gulf veterans.

METHODOLOGY: A population-based health survey. Several studies are currently underway that investigate the incidence, prevalence, and nature of illnesses in Gulf War veterans. The National Health Survey of Persian Gulf Veterans and Their Family Members is conducted in three phases. In this Phase III, the same 1,000 veteran respondents and their family members from each group will be invited to participate in a comprehensive physical examination under a uniform

comprehensive clinical examination protocol at a VA medical facility. Phase III will be completed by February 2001.

EXPECTED PROJECTS (MILESTONES): Publication in a peer-reviewed journal.

STATUS/RESULTS TO DATE: Phase III is in process.

PUBLICATIONS: none to date

Title: Use of Roster of Veterans Who Served in Persian Gulf Area

Project #: VA-3

Agency: VA/DoD

Study Location: VAMC Washington DC

Project Status: Complete

Research Type: Epidemiology Research

P.I.: Han K. Kang, Dr. P.H.

Research Focus: Symptoms/General Health

Start Date (CY): 1994

Est. Completion (CY): 1998

OVERALL PROJECT OBJECTIVE: To properly address the issue of possible exposure to environmental hazards, all individuals who served in the Persian Gulf region need to be identified.

SPECIFIC AIMS: To prepare a computer file of over 697,000 troops assigned to the Persian Gulf area during Operation Desert Shield/Desert Storm.

METHODOLOGY: Armed Services Center for Research of Unit Records is in the process of computerizing troop unit locations in the Persian Gulf Theater.

EXPECTED OUTCOMES (MILESTONES): Complete description of unit locations in the Persian Gulf Theater.

STATUS/RESULTS TO DATE: The personnel database is now operational (about 750,000 individuals). Troop movement software is now operational. Documenting troop movements from Combat Records and from individuals who served in the Gulf is continuing. This database has been made available to VA's Environmental Epidemiology Service (EES), giving VA access to demographic data on troops stationed in the Persian Gulf. The risk assessment data are obtained, and oil field emissions modeling data are synthesized.

RESULTS: The initial troop roster and troop unit locations were available July 1996.

PUBLICATIONS: none to date

Title: Boston Environmental Hazards Research Center Program

Project #: VA-4Core

Agency: VA

Study Location: VAMC Boston

Project Status: **Research Type:** Clinical Research

P.I.: Ozonoff, M.D./ White, Ph.D.

Research Focus: Symptoms/General Health, Brain & Nervous System

Start Date (CY):

Est. Completion (CY): 0

This is the parent Program for VA projects VA-4A through VA-4F. The primary purpose of the BEHC Core is to support pilot projects concerned with PGW veterans' health issues, and thus, provides funding to initiate projects that then will be developed into externally funded projects. Additionally, there are various administrative and organizational tasks that are central to the Center's function.

Therefore, the Core budget reflects the above-described purposes. Specifically, it supports pilot or new start-up projects not currently part of one of the Center's six formal projects, administrative expenses, and partial salary support for personnel who work on specific tasks central to the Center functioning. These tasks include administration (i.e., budget work, coordinating reports

and annual updates), computer set-up and data management, and consultation. Specifics (FYI) Over the past 3+ years, specifically the Core Consulting Budget has supported fees to scientific consultants (~2K/year) and pilot work and salary support for Les Boden Ph.D. for his study of the economic consequences of PGW service (~ 15K/year). The Core Administrative Budget has covered salary support for the Center Secretary (all years) and Administrative Officer (from ~ May 1996 through Jan. 1997), and a portion of the Assistant Director's salary (~40% of salary paid by the Center; the remaining portion of this person's salary paid by the Center was distributed between the 6 Formal projects).

	FY95	FY96	FY97	FY98
Core Personnel**	109,036	127,668	117,201	21,794
Consultants**	16,443	15,944	16,944	4,300
Courses/Mtg Registr.	1,718	3,833	1,062	560
Computer/Equipment	55,410	1,731	13,856	0
Supplies	9,261	3,091	2,841	1,000
Travel	0	0	3,000	500
Tax (3%)	17,949	15,000	15,000	3,750

** Personnel in the Core include salaries for Statistician, Data Manager, Secretary, Administrative Officer, and ~40% Assistant Director.

PUBLICATIONS: see VA-4 subprojects

Title: Boston Environmental Hazards Research Center Program

Project #: VA-4

Agency: VA

Study Location: VAMC Boston

Project Status:

P.I.: Ozonoff, M.D./ White, Ph.D.

Start Date (CY):

Est. Completion (CY): This is the parent program for Projects VA-4A through VA-4F.

PUBLICATIONS: See Projects

Title: Evaluation of Cognitive Functioning of Persian Gulf Veterans

Project #: VA-4A

Agency: VA

Study Location: VAMC Boston

Project Status: Ongoing

Research Type: Clinical Research

P.I.: Jessica Wolfe, Ph.D

Research Focus: Brain & Nervous System, Symptoms/General Health

Start Date (CY): 1994

Est. Completion (CY): 1999

OVERALL PROJECT OBJECTIVE: The primary focus of this research project is to determine whether specific cognitive deficits or deficit patterns are associated with the presence of self-reported health complaints and/or with hazardous environmental exposures in the Gulf.

(Exposure information will be obtained either from self-report, exposure modeling, and DoD data on troop locations). In examining these associations, the influence of war-zone stress, PTSD symptomatology, psychological distress, and post-deployment life stressors is being evaluated.

SPECIFIC AIMS: The specific objectives of this study are: (1) to observe and determine whether cognitive performance deficits are associated with self-reported health complaints stemming from Persian Gulf deployment; (2) to determine the relationship of environmental exposure (self-report and based on an independent exposure protocol) during deployment to cognitive performance; (3) to characterize subsets of cognitive patterns or deficits among exposure classes; (4) to delineate the association of cognitive alterations or deficits with levels of combat exposure, combat stress, and PTSD symptomatology; and (5) to conduct a preliminary examination of the relationship among health symptoms, hazardous environmental exposure, and other war-zone experiences, specifically traumatic war-time stressors and ensuing trauma symptomatology (Sutker, Uddo, Brailey, & Allain, 1993; Wolfe, Keane, & Young, in press). Identification of diagnostic predictors and their relationship to outcomes of interest will help establish preliminary models related to important components of the military experience. The development of these models will aid Department of Veterans Affairs (VA) and Department of Defense (DoD) activities related to veterans' health care by: (1) facilitating symptom recognition and diagnosis in areas of growing concern (environmental exposure and traumatic stress following war), (2) emphasizing interdisciplinary approaches, (3) contributing to treatment planning, and (4) providing a baseline against which cognitive and mental status change over time can be gauged. Observations and findings from this study can lead to more focused investigation of cognitive functions that may be directly associated with defined exposures and/or PTSD symptomatology.

METHODOLOGY: Subjects in this face-to-face study are contacted by phone and asked to participate in as many parts of the protocol as they would like. The protocol consists of an in-depth questionnaire which asks about background information relating to job, education, family history, medical and psychological problems, an interview with an Environmental Health specialist about experiences and exposures while in the Gulf, a psychological interview with a clinical psychologist asking about readjustment issues and psychiatric stress since the war, and neuropsychological tests examining primarily memory, attention, and learning abilities.

EXPECTED PRODUCTS (MILESTONES): Multiple scientific manuscripts, annual reports, annual Reports of Boston Environmental Hazards Center, longitudinal data on stress and health.

STATUS/RESULTS TO DATE: We completed the data collection phase of Project #1 in the Fall of 1996. A total of 220 subjects from a stratified, random sampling from the larger Devens cohort participated. In addition, 73 PGW veterans from the New Orleans area (in collaboration with Drs. Pat Sutker and Jennifer Vasterling); and 50 controls (veterans deployed to Germany but not the Gulf) have completed the Project #1 protocol.

Cleaning and merging all the Project #1 data has been completed and data analyses and manuscript writing are in progress. Analyses of health symptom reporting by the Devens, New Orleans, and Germany-deployed subjects have been carried out specifically examining the effect of self-reported environmental exposure on health symptom reporting, controlling for war-zone exposures and traumatic stress. A manuscript has been written describing these results and it has been submitted for consideration for publication. In addition, a "Letter to the Editor" to the American Journal of Epidemiology (in response to a recent article by Dr. Haley about PTSD) has been accepted for publication and is in press. Several other manuscripts are in progress (topics include: neuropsychological test performance, PTSD and psychiatric status, and exposure

assessment). (A paper describing health symptom reporting in the Devens cohort at Time 2 was recently published in the American Journal of Industrial Medicine.)

In order to substantiate and corroborate information reported to us by veterans regarding potential hazardous exposures in the Gulf, we are working on incorporating all the collected epidemiological data into a Geographic Information System (GIS) format so that temporal and spatial characteristics can be modeled and integrated into data analytic procedures. We are also working with the US Army's Center for Health Promotion and Preventative Medicine (CHPPM) and the US Army's Center for Research of Unit Records (RUR) to incorporate some of their information on oil fire smoke exposure estimates and unit-level troop locations into our analyses. When we have worked out the technical issues regarding modeling location information, we will conduct analyses to compare individual troop location and exposure information from our study subjects to information contained in the RUR computer database for our particular units of study and their effect on health symptom patterns, neuropsychological test performance, and psychological stress.

PUBLICATIONS: Proctor SP, Heeren T, White RF, Wolfe J, Borgos MS, Davis JD, Pepper L, Clapp R, Sutker PB, Vasterling JJ, Ozonoff D. Health status of Persian Gulf War veterans: self-reported symptoms, environmental exposures and the effect of stress. *Intl J Epidemiol*, 1000-10, 1998.

Wolfe J, Proctor SP, Davis JD, Borgos MS, Friedman M. Health symptoms reported by Persian Gulf War veterans two years after return. *Am J Ind Med*, 33:104-113, 1998.

Wolfe J, Proctor SP, White RF, Friedman M. Letter to the Editor. *Am J Epidemiol*, 148:402, 1998.

Yokoyama K, Araki S, Murata K, Nishikitani M, Okumura T, Ishimatsu S, Yakasu N, White R. Chronic neurobehavioral effects of Tokyo subway sarin poisoning in relation to Posttraumatic Stress Disorder. *Arch Environ Health*, 53:249-256, 1998.

Wolfe J, Proctor SP, Erickson DJ, Heeren T, Friedman MJ, Huang MT, Sutker PB, Vasterling JJ, White RF. Relationship of post-traumatic stress disorder and depression to Gulf War veterans' health problems. Manuscript submitted for publication to *Psychosomatic Medicine*.

White RF, Proctor SP, Heeren T, Wolfe J, Ozonoff D, Krenzelok M, Lindem K, Vasterling JJ, Sutker PB, Heaton, K. Neuropsychological function in Gulf War veterans: Relationships to self-reported toxicant exposures. Manuscript in preparation.

Proctor SP, Heeren T, Wolfe J, White RF, Ozonoff D. Analysis of Persian Gulf War veterans near Khamisiyah, Iraq in early March 1991. Manuscript in preparation.

Proctor SP, Harley R, Wolfe J, Heeren T, White R. Functional Status (SF-36) of Gulf War Veterans. Manuscript in preparation.

Proctor SP, White RF, Wolfe J, Heeren T, Borgos MS, Davis JD, Vasterling J, Sutker P. Health status of Persian Gulf War veterans: Self-reported health symptoms. Presented as part of a symposium "Persian Gulf War Veterans' Health Complaints- Psychological Studies" at the Annual Meeting of the American Psychological Association, Chicago, IL; Organizer: RF White, Ph.D., August 18, 1997.

White RF, Krenzelok M, Proctor SP, Wolfe J, Heeren T, Borgos MS, Davis JD, Pepper L, Vasterling JJ, Sutker PB, Ozonoff D. Neuropsychological findings among Persian Gulf-War era veterans. Presented at the Conference on Federally Sponsored Gulf War Veterans' Illnesses Research, Pentagon City, VA; June 1998

Title: Evaluation of Neurological Functioning in Persian Gulf Veterans

Project #: VA-4B

Agency: VA

Study Location: VAMC Boston

Project Status: Complete*

Research Type: Clinical Research

P.I.: Robert Feldman, M.D.

Research Focus: Brain & Nervous System

Start Date (CY): 1994

Est. Completion (CY): 1999 Symptoms/General Health

OVERALL PROJECT OBJECTIVE: This study evaluates neurological functioning in returning Persian Gulf War (PGW) veterans using clinical examinations and neuroimaging techniques capable of detecting subtle changes in central nervous system functioning (functional magnetic resonance imaging [fMRI]). The following study description reflects a change from the original proposal.

SPECIFIC AIMS: The specific objectives of this study are: (1) to objectively describe Persian Gulf War veterans' neurological status using standard clinical methodology, (2) to observe and determine patterns of central nervous system dysfunction in PGW veterans and PGW-era veterans and (3) to explore whether similar patterns of particular exposure characteristics exist within the groups and whether distinct patterns exist between the groups.

METHODOLOGY: Subjects for clinical neurological exams are veterans from the subjects evaluated in Project 1. These subjects are also being examined as part of the laboratory and pulmonary function study in Project #4, VA-4D.

EXPECTED PRODUCTS (MILESTONES): In this study, we expect to determine if there are differences in subtle neurological functioning between PGW veterans and PGW-era veterans.

STATUS/RESULTS TO DATE: Fifty subjects have undergone neurological exams. Pilot functional magnetic resonance imaging (fMRI) studies are currently in progress. This past year has been devoted to establishing the paradigms and setting up the equipment necessary to perform and analyze the functional imaging studies, as well as to begin scanning normal controls. Preliminary neuropsychological testing (from Project #1) has revealed several areas of behavior where these subject groups differ from normal controls. One of those areas is working memory. We have therefore created a visual working memory task that can be used for fMRI studies. In addition, we will examine motor function (Finger Tapping) and attention (Continuous Performance Test) in fMRI studies. We have also completed the computer programs necessary to analyze the fMRI imaging studies.

In the summer of 1997, we submitted a grant application to establish a collaborative agreement project with CDC in which we used the preliminary information collected on fMRI (described above) and the work done on establishing the test paradigms to enlarge the scope of the above described fMRI project. This new study project was funded on November 1, 1997 (for 3 years). Thus, the fMRI work will now be incorporated into the new CDC grant's scope. This project is now complete and information has been incorporated into Project # HHS-5.

PUBLICATIONS: none to date

Title: Gulf War And Vietnam Veterans Cancer Incidence Surveillance

Project #: VA-4C

Agency: VA

Study Location: VAMC Boston

Project Status: Ongoing

Research Type: Epidemiology Research

P.I.: Richard Clapp, D.Sc.

Research Focus: Environmental Toxicology

Start Date (CY): 1994

Est. Completion (CY): 1999

OVERALL PROJECT OBJECTIVE: To examine the pattern of cancer diagnosed in Massachusetts Vietnam veterans as compared with Vietnam-era veterans for the time period 1988-1993. This linkage will be accomplished by linking a computer tape of Massachusetts veterans who received differential bonuses to the Massachusetts Cancer Registry master file and analyzing the resulting linked cases, as was done for the years 1982-1988 in prior study by the Principal Investigator. Another objective is to establish a database linkage for New England area

Persian Gulf War veterans so as to be able to examine cancer incidence in this cohort at a later point in time.

SPECIFIC AIMS: 1. Monitor cancer incidence in Gulf War and Vietnam veterans of New England states, as part of the overall surveillance of disease in veterans exposed to environmental hazards; 2. Extend previous studies of cancer incidence and mortality by collecting and analyzing data on Massachusetts Vietnam veterans diagnosed in the years 1988-1993; 3. Analyze the results of the Massachusetts veterans cancer incidence linkage in a nested case-control design incorporating information on military and civilian occupations, branch of service and, where possible, location of service; 4. Establish a roster of Gulf War veterans in New England states, with identifying information such as names, dates of birth, and social security numbers, resulting in a computer file of Gulf War veterans that can be used in future linkage studies examining cancer incidence and mortality from various diseases; 5. Provide a database from which to plan additional clinical and preventative interventions to reduce morbidity and mortality from targeted diseases.

METHODOLOGY: In Year 1, we initiated the linkage. In years 2-5, we sorted the cases into cancers of interest and other (auxiliary) cancers, analyzed the data as a "nested" case-control study, and present odds ratios for specific cancers in Vietnam veterans compared with Vietnam-era veterans. Also in years 2-5, we devised a system for linking the roster of Gulf War veterans in the New England states with cancer registries in all states which have population-based data.

EXPECTED PRODUCTS (MILESTONES): In this study we expect to determine cancer incidence rates in Massachusetts Vietnam veterans for the period 1988-1993. At completion of year 2 of the study, we expect to have preliminary results based on analysis of this database. Results pertaining to cancer incidence in Gulf War veterans is not expected for 10 years or so due to the latency period in cancer development.

STATUS/RESULTS TO DATE: Dr. Clapp has obtained the agreement of all the New England Cancer Registry Directors to proceed with the Gulf War Veterans linkage study. Additionally, Dr. Clapp has contacted the staff at Defense Manpower Record System to determine the specific data request and confidentiality requirements for obtaining the roster of New England Gulf War veterans. In the upcoming months, Dr. Clapp will submit the required forms and assurances to the Boston University Institutional Review Board. Once approval has been granted, the record linkage process will be undertaken. Dr. Clapp has completed his update of cancer incidence in Massachusetts Vietnam veterans compared to Vietnam-era veterans. The study concluded that there were substantially increased diagnosed between the ages of 35 and 64 during the years 1988 to 1993, there were substantially increased numbers of nasal/nasopharyngeal cancer and oral cancer of the 245 cancers in Vietnam veterans diagnosed between the ages of 35 and 64 during the years 1988 to 1993. A Letter to the Editor to the International Journal of Epidemiology describing these findings has been published.

PUBLICATIONS: Clapp, R. Update of cancer surveillance of veterans in Massachusetts.

Letter to the Editor, International Journal of Epidemiology; 26(3):679-681, 1997.

Clapp R. Cancer surveillance of veterans in Massachusetts 1988-1993. Abstract presented at the North American Association of Central Cancer Registries, 1997

Clapp R. Cancer surveillance of Vietnam veterans in Massachusetts, USA 1982-1993.

Abstract submitted for presentation at the 12th Symposium of the Scientific Committee on Epidemiology in Occupational Health of the International Commission on Occupational Health held in Zimbabwe, September 16-19, 1997. Annual Conference held in Boston, MA, April 1-3, 1997.

Title: Evaluation of Respiratory Dysfunction Among Gulf War Veterans

Project #: VA-4D

Agency: VA

Study Location: VAMC Boston

Project Status: Ongoing

Research Type: Clinical Research

P.I.: Lewis Pepper M.D.

Research Focus: Environmental Toxicology, Symptoms/General Health

Start Date (CY): 1994

Est. Completion (CY): 2001

OVERALL PROJECT OBJECTIVE: To assess respiratory status in relation to exposure to oil fire pollution in Gulf War veterans and evaluate the relationship between measures of pulmonary dysfunction and respiratory symptoms and exposure in subjects recruited for Project #1.

SPECIFIC AIMS: 1. Create a cumulative personal exposure estimate for each subject utilizing the U.S. Army Center for Health Promotion and Preventive Medicine (USACHPPM) exposure assessment data for their unit combined with geographic specific information which maps each unit's location in the Persian Gulf War theater; 2. Assess respiratory status using standardized respiratory questionnaire; 3. Assess respiratory function with physical examination and pulmonary function testing; 4. Assess pre-existing asthma risk with total serum IgE levels; 5. Assess non-specific bronchial responsiveness (BHR) as a measure of early outcome and as a potential risk factor in the development of lung disease by using methacholine challenge testing (initiating in Year 2 of the study); 6. Examine possible predictors of respiratory function (pulmonary function tests (PFT) and methacholine challenge test) using cumulative environmental exposure assessment variable, respiratory medical history, occupational history, and smoking status variables; 7. Develop a database from which to plan preventative interventions to reduce morbidity and mortality from respiratory diseases.

METHODOLOGY: Subjects will be veterans from the subjects evaluated in Project 1. Each will complete a standardized respiratory questionnaire, systematic cardio-pulmonary physical exam, and pulmonary function testing. Spirometric measures will include forced expiratory volume in one second (FEV1), forced vital capacity (FVC) and FEV1/FVC in accordance with American Thoracic Society recommendations. Subject data will be adjusted for age, height and gender and transformed to percentage predicted values using Knudson predictive equations. Blood will be drawn by venapuncture at the time of the visit and total serum IgE, complete blood count and differential and quantitative immunoglobulin levels will be measured. Bronchial responsiveness will be determined via methacholine challenge testing. Nonspecific bronchial hyperresponsiveness has been suggested as a risk factor for, or an early event in the development of both acute and chronic occupational lung diseases. Occupational exposures to irritant substances at concentrations similar to the Gulf War exposures have been shown to cause airway hyperresponsiveness and it is believed that these exposures might be associated with the development of asthma.

EXPECTED PRODUCTS (MILESTONES): The project will assess pulmonary outcome measures, assign exposure measures and examine the relationship between exposure and outcome measures. Outcome measures will be assessed using a standardized respiratory questionnaire, and pulmonary function tests. Bronchial hyper-responsiveness will be assessed as a measure of early outcome and as a potential risk factor in the development of lung disease. Exposure measures include self-reported exposure to smoke from oil well fires as well as modeled exposure estimates. We will examine the potential predictors of respiratory function using exposure assessment variables, respiratory medical history, and a smoking status variable. At the completion of Year 4, a preliminary case series analyses of the data will be conducted to examine associations between reported oil well fire exposure and respiratory status.

STATUS/RESULTS TO DATE: The study protocol was expanded to include members of a unit in an air ambulance company who were activated and sent to Germany during the Gulf War during December 1990 through August 1991. This group was added as a comparison group as it was mobilized but not deployed to the Persian Gulf. It consists of medics, helicopter pilots, flight crews, mechanics, communications specialists, and administrative support personnel whose intended mission was to handle and transport wounded US soldiers evacuated from the Gulf. Due to low US military casualties, the unit assisted German civilian missions. Fifty subjects from this unit have participated in Project #1. In order to conduct the methacholine challenge test for bronchial hyper-responsiveness, the study test site has been relocated to the Pulmonary Lab at

Boston Medical Center. At this time a total of 89 pulmonary function tests have been given. Sixty-seven subjects completed conventional pulmonary function testing, respiratory and environmental questionnaires during the first phase of the study. An additional 22 subjects have completed the methacholine challenge tests, conventional function testing, respiratory and environmental questionnaires as part of the second phase of the study. Thirteen of the 67 participants tested during the first phase have returned to participate in the second phase of the study (methacholine challenge testing). To complete the study, transition funding has been requested for FY00 and FY01. It is anticipated that the study will be complete by FY01.

PUBLICATIONS: Stinson MC, Pepper L. An evaluation of respiratory function among Persian Gulf War veterans. Presented as a Poster at the Conference on Federally Sponsored Gulf War Veterans' Illnesses Research, Washington, DC June 17, 1998.

Title: The Aromatic Hydrocarbon Receptor (AhR) as a Biomarker of Susceptibility

Project #: VA-4E

Agency: VA

Study Location: VAMC Boston

Project Status: Ongoing

Research Type: Mechanistic

P.I.: David Sherr, Ph.D.

Research Focus: Environmental Toxicology, Prevention

Start Date (CY): 1994

Est. Completion (CY): 1999

OVERALL PROJECT OBJECTIVE: To test the hypothesis that the aromatic hydrocarbon receptor (AhR) is a molecular biomarker for polycyclic aromatic hydrocarbon (PAH) susceptibility. Veterans of the Persian Gulf war were potentially exposed to relatively high doses of PAHs in smoke emanating from Kuwaiti oil well fires. The high incidence of physical complaints by Gulf War veterans may reflect previously documented PAH toxicities including suppression of the immune system and neurologic dysfunction.

SPECIFIC AIMS: 1. Confirm that AhR expression levels correlate with PAH bioactivity. 2. Optimize the use of single strand conformational polymorphism (SSCP) analysis to detect AhR gene polymorphism and mutations in mice. 3. Apply results to human studies. Results obtained in the fulfillment of aims 1 and 2 will define the conditions for employing AhR levels and gene polymorphism as molecular biomarkers of PAH susceptibility.

METHODOLOGY: For the first aim, initial experiments require molecular genetic manipulation to produce strains of mice expressing various levels of the AhR. AhR gene constructs have been made and tested both in vitro and in vivo. Analysis of the effects of ectopic AhR expression require cellular biology techniques including flow cytometry, cell culture and analysis of the immunologic competency of lymphocytes in vivo and in vitro. For human studies, we have optimized the use of single strand conformational polymorphism (SSCP) analyses. DNA is extracted from peripheral blood lymphocytes from Persian Gulf War veterans who complete Projects 1 and 4. Double stranded DNAs from these samples are separated by heating followed by rapid cooling on ice. The three-dimensional conformation reassumed by each strand is determined by its nucleic acid sequence. Single nucleotide changes can be detected when the reannealed DNAs are electrophoresed on 6% acrylamide gels. Samples showing variant banding patterns are cloned and sequenced to compare putative AhR alleles.

EXPECTED PRODUCTS (MILESTONES): The production of new strains of mice which differ in their responsiveness to environmental pollutants. These mice will represent invaluable tools in investigating the effects of these pollutants in humans. These studies have also demonstrated that high level AhR expression is a biomarker for malignant cell transformation. This observation have led to experiments designed to test the role of the AhR in tumorigenesis and to evaluate the

effect on AhR inhibitors on human tumor growth.

STATUS/RESULTS TO DATE: The primary goal of aim # 1 is to develop mutant mice which express various levels of AhR. We have generated a colony of AhR gene knock-out mice and have evaluated their responses to PAH. Results thus far demonstrate that PAH induce apoptosis of immature B lymphocytes in vivo and in vitro and that this form of immunotoxicity is dependent on the AhR expressed in stromal cells of the lymphopoietic microenvironment and not in immature B cell themselves. Using a murine AhR cDNA clone regulated by the immunoglobulin enhancer/promoter region, we have produced and analyzed AhR transgenic mice. Transgenic mice from at least 2 founders express high levels of the AhR in both the thymus and spleen. Histologic and flow cytometric analyses of these organs indicate a severe dysregulation of T cell maturation including expression of CD4+/CD8+ T cells in lymph nodes suggesting that the AhR plays a critical role in T cell development. Experiments now being carried out are designed to evaluate the mechanisms of this developmental defect. In addition, we will evaluate more closely the potential for ectopic AhR expression to influence B lymphocyte development and function. The purpose of specific aim #3 is to determine if human AhR polymorphism exist. We have now evaluated over 50 Persian Gulf War veterans using AhR-specific DNA primers to amplify biologically important sections of their AhR genes. Using single strand conformation polymorphism assays we have screened both the DNA- and ligand-binding domains of the human AhR and found that the AhR is highly conserved in these regions. It is concluded, therefore, that the AhR plays a critical function that cannot be altered by mutation without significantly compromising survival.

PUBLICATIONS: Vaziri C, Shneider A, Sherr DH, Faller D. Expression of the aryl hydrocarbon receptor is regulated by serum and mitogenic growth factors in murine 3T3 fibroblasts. *J Biol Chem*, 271, 25921-25927, 1996.
Yamaguchi K, Near RI, Matulka RA, Shneider A, Toselli P, Trombino AF, Sherr DH. Activation of the aryl hydrocarbon receptor/transcription factor and bone marrow stromal cell-dependent preB cell apoptosis. *J Immunol*, 158:2165-2173, 1997.
Yamaguchi K, Near R, Shneider A, Cui H, Ju S-T, Sherr DH. Fluoranthene induced apoptosis in murine T cell hybridomas is independent of the aromatic hydrocarbon receptor. *Toxicol Appl Pharmacol*, 139:144, 1996

Title: Validity of Computerized Tests

Project #: VA-4F

Agency: VA

Study Location: VAMC Boston

Project Status: Ongoing

Research Type: Clinical Research

P.I.: Roberta White, Ph.D.

Research Focus: Brain & Nervous System, Diagnosis

Start Date (CY): 1994

Est. Completion (CY): 1999

OVERALL PROJECT OBJECTIVE: The objective of this project is to continue the validation process of the Neurobehavioral Evaluation System (NES), a battery of computer-assisted behavioral tests developed for the purpose of detecting brain dysfunction secondary to exposure to neurotoxicants, with an expanded battery of subtests. The NES tests are derived from standard neuropsychological tests that have been well characterized in terms of brain-behavior relationships, but differ in input and output modality and in other respects from traditional tests. Therefore, the validity of the NES subtests can only be verified by testing patients with known brain damage and showing that such damage affects performance on those tests. The sensitivity of the NES to neurotoxicant exposure cannot be fully interpreted, in terms of CNS mechanisms, without such validation. Because separate funding was received for a wide scale validity study of

the NES3 (HSR&D Cooperative Study #29, PI: Roberta F. White, Ph.D.), this project has been curtailed to include one especially relevant clinical group for effects of toxicants, patients with Parkinson's Disease (PD). This reflects a change from the original proposal.

SPECIFIC AIMS: 1. Assess the validity of the expanded battery of NES tests within a patient group with Parkinson's Disease) studied in the previous phase of this study. 2. Correlate performance on the new NES subtests with performance on the standard paper-and-pencil tests on which they are based. 3. Determine the sensitivity of the NES subtests to the progression of neurologic disease by longitudinal testing of PD patients.

METHODOLOGY: Subjects are being recruited from both the Boston VAMC and Boston University Medical Center. Each subject will have an initial interview to provide demographic data followed by administration of the Mini-Mental Status Examination and WAIS-R Information and Picture Completion subtests (40 minutes). Subjects meeting inclusion criteria will then be administered the expanded NES battery (approximately 100 minutes).

EXPECTED PRODUCTS (MILESTONES): This study has important clinical and research implications: (1) to facilitate the assessment of patients with suspected subtle brain damage secondary to exposure from neurotoxicants by providing a sensitive, relatively brief battery of tests of CNS function that can be easily administered; (2) to provide a test battery for which brain-behavior relationships are known (providing measures which will be useful in hypothesis-driven research on the cerebral localization of neurotoxicant effects); (3) to provide tests with known diagnostic validity for application to clinical situations.

STATUS/RESULTS TO DATE: Data analysis and manuscript writing have proceeded describing the validity of NES2 in various patient groups. Two papers have been written and published. The NES3 battery fully has been developed. Subject recruitment is ongoing and data collection will be completed in 1999.

PUBLICATIONS: Kregel M, White RF, Diamond R, Letz R, Cyrus P, Durso R. A comparison of NES2 and traditional neuropsychological tests in a neurologic patient sample.

Neurotoxicology and Teratology, 18:435-439, 1996.

White RF, Diamond R, Kregel M, Lindem K, Feldman RG: Validation of the NES2 in Patients with Neurologic Disorders. Neurotoxicology and Teratology, 18:441-448, 1996.

Title: East Orange Environmental Hazards Research Center Program

Project #: VA-5Core

Agency: VA

Study Location: VAMC East Orange, NJ

Project Status: Ongoing

Research Type: Clinical Research

P.I.: Natelson, M.D./ Ottenweller, Ph.D.

Research Focus: Symptoms/General Health, Brain & Nervous System

Start Date (CY):

Est. Completion (CY): 0

This is the parent Program for VA projects VA-5A through VA-5D. NJCEHR funds that support all center projects, rather than specific ones, are allocated under the "CORE". This includes salaries for the Administrator, Scientific Coordinator, Statistician, Statistical Aide, Biomedical Engineers, Systems Analyst, and Associate Research Director. The support of these staff members is required for all Center projects. In addition, core funds cover miscellaneous expenses such as investigator travel, subject reimbursement, subject meals and office supplies & equipment. The percentages of these costs that are allocated for each project varies according to which projects are active during each fiscal year. During this year, approximately 66% of these resources are devoted to Project 3. Thirty-four percent is devoted to a continuation of Project 2. The only major deviation from this allotment of resources is that the Statistician and Statistical Aide are currently devoting their time to analyses of data generated in project 2.

PUBLICATIONS: see VA-5 subprojects

Title: East Orange Environmental Hazards Research Center Program

Project #: VA-5

Agency: VA

Study Location: VAMC East Orange

Project Status:

P.I.: Natelson, M.D./ Ottenweller, Ph.D.

This is the parent Program for VA projects VA-5A through VA-5D.

PUBLICATIONS: See EOEHRC Projects

Title: Health and Exposure Survey of Persian Gulf Veterans

Project #: VA-5A

Agency: VA

Study Location: VAMC East Orange

Project Status: Ongoing

Research Type: Epidemiology Research

P.I.: Howard Kipen, M.D., MPH

Research Focus: Symptoms/General Health

Start Date (CY): 1994

Est. Completion (CY): 1999

OVERALL PROJECT OBJECTIVE: To survey 2800 Persian Gulf War registry veterans randomly sampled from the Northeast quadrant of the U.S. to determine risk factors and their relationships to symptom clusters that could represent significant health problems for Gulf veterans.

SPECIFIC AIMS: 1. To provide health and medical histories of registry veterans who served in the Persian Gulf; 2. To characterize the medical and combat experiences of these veterans during the Gulf war; 3. To perform case control comparisons for various categories and combinations of symptoms with respect to individual and environmental risk factors identified in Aims 1 and 2.

METHODOLOGY: The survey was mailed from Environmental Epidemiology Service to veterans who served in the Gulf. This survey was mailed to 2800 veterans on the Persian Gulf Registry, including 1,935 selected at random from the registry. This survey includes a symptom checklist, an environmental risk factor survey, a family and social support survey, and anxiety and depression scales. Multivariate analysis of the resulting information will include cluster analysis, principal components factor analysis, and multidimensional scaling. From these analyses we expect to be able to develop one or more case definitions for the unexplained illnesses afflicting Persian Gulf veterans.

EXPECTED PRODUCTS (MILESTONES): We have found a high prevalence of Chronic Fatigue and Chemical Sensitivity among registry veterans. We also expect to delineate a series of risk factors for the development of these illnesses, and for Post-traumatic Stress Disorder which may cluster with one or both of these illnesses.

STATUS/RESULTS TO DATE: Data collection is complete. We have received 60% of our questionnaires back, including 1,161 (60%) from the random group. Symptom data has been preliminarily analyzed. The mean number of endorsed symptoms out of 48 total was 24, with headaches, difficulty remembering, and fatigue being the most bothersome. Factor analysis has identified five factors that characterized those in poor health and a preliminary definition has been presented. Confirmatory factor analyses are now being done, and case control analyses will follow.

PUBLICATIONS: Hodason M. Kipen H. Puolisi M. Gulf War illness: causation and treatment.

Journal of Occupational and Environmental Medicine; Accepted, pending revisions
Kipen, Hallman, Kang, Fiedler, Natelson. Prevalence of Chronic Fatigue and Chemical Sensitivities in Gulf Registry Veterans. Submitted to Archives of Environmental Health.
Hallman W, Kipen H, Diefenbach M, Kang H, Wartenberg D, Fiedler N, et al. Defining Gulf War illness: self-reported health status among VA registry veterans. Conference on Federally Sponsored Gulf War Veterans' Illnesses Research, Washington DC. 1998.
Kipen H, Hallman W, Diefenbach M, Kang H, Fiedler N, Natelson B. Self-reported health status and exposure among Gulf War registry veterans. American Public Health Association Annual Meeting, Indianapolis, IN 1997.
Kipen H, Hallman W, Diefenbach M, Kang H, Wartenberg D, Fiedler N, et al. Self-reported health status and exposure among Gulf War Registry veterans. ISEE/ISEA Joint Conference 1998.

Title: Physiological and Psychological Assessments of Persian Gulf Veterans

Project #: VA-5B

Agency: VA

Study Location: VAMC East Orange

Project Status: Complete

Research Type: Clinical Research

P.I.: Benjamin Natelson, M.D.

Research Focus: Immune Function, Brain & Nervous System

Start Date (CY): 1994

Est. Completion (CY): 1998

OVERALL PROJECT OBJECTIVE: This project will determine if some of the unexplained medical illnesses in Persian Gulf veterans may be similar to Chronic Fatigue Syndrome (CFS) or Multiple Chemical Sensitivity (MCS) in civilian populations. It will include viral/immunological studies, neuropsychological studies, and studies of physiological responses to a number of physical and cognitive challenges. A series of psychosocial factors will be evaluated to determine whether they are risk factors for the illnesses suffered by these veterans.

SPECIFIC AIMS: 1. To determine whether viral or cytokine patterns may suggest an infectious agent as a cause of illness in Persian Gulf veterans; 2. To determine if these veterans suffer chronic fatigue or chemical sensitivity; 3. To determine psychosocial risk factors that might have predisposed some veterans to develop their medical problems; 4. To determine if there are neuropsychological deficits in Persian Gulf veterans; 5. To determine if these veterans have abnormal physiological responses to physiological and cognitive challenges.

METHODOLOGY: Persian Gulf veterans who have chronic fatigue, chemical sensitivity, or a combination of these and healthy control veterans are recruited for these studies. Blood samples are drawn, peripheral lymphocytes are isolated, and polymerase chain reactions are used to quantify herpes, Epstein-Barr and other viruses and to assess the level of interleukins and tumor necrosis factor-alpha. The subjects fill out a series of psychosocial instruments and environmental exposure surveys. A battery of neuropsychological tests are given in conjunction with magnetic resonance imaging of the brain. To evaluate the possibility of early peripheral neuropathy, neurological evaluation is supplemented with test of threshold for fine touch in the distribution of the radial nerve in the hand using Semmes-Weinstein monofilaments. Finally, we perform comprehensive testing of autonomic nervous system function and evaluate the veteran's ability to respond appropriately to behavioral challenges.

EXPECTED PRODUCTS (MILESTONES): We expect a number of the unexplained illnesses in Persian Gulf veterans will be similar to CFS and MCS. Data on civilians with CFS suggest that some cytokines and some of the herpes viruses might be elevated. Our own data on civilians with CFS reveal neuropsychological deficits in memory tasks and occasional non-specific white matter changes in the MRI's. Finally, we expect abnormal responsiveness to some of the physiological stimuli, but it remains unclear whether there will be hyper- or hyporesponsiveness.

STATUS/RESULTS TO DATE: This project ended last March. Data continue to be summarized

and prepared for publication, and we have had our first papers accepted. New results include the following: 1) There is no evidence of herpesvirus reactivation to explain the symptoms of Gulf vets with CFS. 2) However, these vets do show evidence of immune dysregulation (i.e. increases in T cells and decreases in NK cells) which we did not find in civilians with CFS. 3) Contrary to Haley's statement that PTSD is rare in Gulf vets, we found a considerable number of veterans afflicted with this problem, with many of them reporting classic war related traumatic events. 4) Both Gulf vets and civilians with the CFS have subtle abnormalities on quantitative sensory testing.

PUBLICATIONS: Fiedler N, Kipen H, Natelson B, Ottenweller J. Chemical sensitivities and the Gulf War: Department of Veterans Affairs Research Center in Basic and Clinical Studies of Environmental Hazards. *Regulatory Toxicology and Pharmacology* 1996; 24:S129-138.

Fiedler N, Kipen H. Multiple Chemical Sensitivity, Chronic Fatigue Syndrome, and the Gulf War. *Toxicology and Industrial Health* 1998; In press.

Pollet C, Natelson B, Lange G, Tiersky L, DeLuca J, Policastro T, et al. Medical evaluation of Persian Gulf veterans with fatigue and/or chemical sensitivity. *Journal of Medicine*, 29(3&4):101, 1998.

Tiersky L, Natelson B, Ottenweller J, Lange G, Fiedler N, DeLuca J. Functional status and mood in Persian Gulf veterans with unexplained fatiguing illness. *Military Psychology* 1998; Accepted, pending minor revisions.

Wallace H, Natelson B, Gause W, Hay J. An evaluation of human herpes viruses in Chronic Fatigue Syndrome. *Clinical and Diagnostic Laboratory Immunology* 1998; In press.

Zhang Q, Zhou X-D, Denny T, Ottenweller J, Lange G, LaManca J, et al. Changes in immune parameters are seen in Gulf veterans but not civilians with chronic fatigue syndrome. *Clinical and Diagnostic Laboratory Immunology*, 6:6-13, 1999.

Fiedler N, Kipen H, Natelson B, Ottenweller J. Chemical sensitivities and the Gulf War: Department of Veterans Affairs Research Center in basic and clinical science studies of environmental hazards. Multiple Chemical Sensitivities: State of the Science Symposium, Baltimore, MD, October 1995.

Fiedler N, Lange G, Tiersky L, DeLuca J, Policastro T, Kelly-McNeil K, et al. Fatiguing illness and the Gulf War veteran: The NJ Environmental Hazards Research Center. 105th Annual Meeting of the APA, Chicago, IL 1997.

Fiedler N, Lange G, Tiersky L, DeLuca J, Policastro T, Kelly-McNeil K, et al. Stress, personality, & coping in Gulf War veterans with fatiguing illness. Conference on Federally Sponsored Gulf War Veterans' Illnesses Research, Washington DC. 1998.

Fiedler N, Kipen H. Multiple Chemical Sensitivity, Chronic Fatigue Syndrome, and the Gulf War. American Chemical Society Symposium, Boston, MA, August 1998

Title: Effects of Exertion and Chemical Stress on Persian Gulf Veterans

Project #: VA-5C

Agency: VA

Study Location: VAMC East Orange

Project Status: Ongoing

Research Type: Clinical Research

P.I.: Nancy Fiedler, Ph.D.

Research Focus: Immune Function, Symptoms/General Health, Environmental Toxicology

Start Date (CY): 1997

Est. Completion (CY): 1999

OVERALL PROJECT OBJECTIVE: The first part of this project will examine the effect of controlled chemical exposure and psychological stress on symptoms and psychophysiologic and cognitive performance of Persian Gulf veterans previously diagnosed with fatiguing illness.

Research in our Center found that relative to healthy veterans, PG veterans with fatiguing illness

reported significantly more combat exposure, illness in response to chemical exposures such as diesel exhaust and greater levels of negative affect and defensiveness. Therefore, this study will test the effects of each of these variables in a controlled study. The purpose of the second part of this project will be to investigate the acute effects of exhaustive exercise on the cognitive abilities and the cardiovascular responses to autonomic stressors in Persian Gulf veterans diagnosed with Chronic Fatigue Syndrome (CFS). To date our data have indicated impaired cognitive functioning and evidence of abnormal cardiovascular reactivity to various laboratory stressors in veterans with CFS when compared to healthy veterans. Also a significant number of the fatigued veterans have indicated extreme fatigue and worsening of symptoms after even moderate physical exertion. An additional variable which will be examined will be cardiopulmonary fitness. In this area we have preliminary data from project VA-5B which indicate a reduced pulmonary function in veterans with CFS, especially those who had indicated that they became sick when exposed to smoke from oil fires in the Gulf War.

SPECIFIC AIMS: 1) To assess the effect of exposure to diesel fumes and psychological stress in the following PG veteran groups: Healthy; Healthy/Negative Affect; Fatiguing Illness/Negative Affect; Fatiguing Illness/Defensive. 2) To assess the effect of exhaustive exercise on the cognitive abilities and cardiovascular response to autonomic stressors of PG veterans with CFS and healthy PG veterans.

METHODOLOGY: Veterans who were previously evaluated at our Center will be recruited. Each veteran will undergo a complete physical and psychiatric examination. Veterans who meet criteria for one of the subject groups will participate in a single experimental session during which symptoms, psychophysiological response (i.e., heart rate, blood pressure, respiration rate, end-tidal CO₂, finger pulse volume, and skin conductance), and performance on a computerized vigilance task will be monitored in response to a rest period, followed by a psychological stressor (Stroop Color Word test), and exposure to 5 ppm diesel fumes. Blood and breath samples will confirm body burden of exposure. For the exhaustive exercise study, PG veterans with CFS and healthy PG veterans who were previously evaluated at our Center will be recruited. The subjects will perform a cognitive test battery. Next they will perform a series of cardiovascular stressors (CVEVAL): 1) supine to standing, 2) Valsalva maneuver and 3) reaction time. In this CVEVAL session we will measure beat-by-beat blood pressure, ECG activity, respiration, and end tidal CO₂. After these sessions the subjects will perform a maximal aerobic capacity test on a bike ergometer (VO₂max). During the VO₂max test metabolic data will be measured breath-by-breath, lactic acid will be assessed from finger stick blood samples, and muscle oxygenation will be evaluated by near infrared spectroscopy. Following the VO₂max test the subjects will repeat the two pre-test sessions.

EXPECTED PRODUCTS (MILESTONES): The exposure study will determine the health effects of an acute exposure to an agent (i.e., diesel), reported by veterans with fatiguing illness, to have caused symptoms during service in the Gulf and presently. The study will also assess the differential contribution of personality variables and stress to subjective (i.e., symptoms) and objective (performance, physiology) indicators of health effects. Regarding the exercise study, research has shown that the added stress of physical exercise can in some cases expose autonomic problems when conventional test have failed. Also data in our civilian population with chronic fatigue have shown a decrease in cognitive abilities after physical exertion when compared to healthy controls. We may see similar results in this veteran population.

STATUS/RESULTS TO DATE: To date, 25 veterans have participated in the diesel exposure study. Relative to healthy controls, CFS subjects show increased symptoms and poor cognitive function during exposure to diesel fumes. Twenty-five veterans have also participated in the study concerned with effects of exercise on the cardiovascular responses to autonomic stressors and cognitive abilities. At this time the group numbers are too small to make definite statements about results. However, the experiments are going well with no adverse effects on the subjects. The sick veterans seem to tolerate the exercise sessions and at this time there does not appear to be an obvious difference in the fitness level of the groups. Our plans are to continue with the experiments.

PUBLICATIONS: none to date

Title: Effects of Genetics and Stress on Responses to Environmental Toxins

Project #: VA-5D

Agency: VA

Study Location: VAMC East Orange

Project Status: Complete

Research Type: Mechanistic

P.I.: John Ottenweller, Ph.D.

Research Focus: Pyridostigmine Bromide, Interactions

Start Date (CY): 1994

Est. Completion (CY): 1997

OVERALL PROJECT OBJECTIVE: This project will determine whether genetic susceptibility to stress and exposure to chronic stress will result in persistent sensitization to potentially toxic effects of pyridostigmine bromide and aromatic hydrocarbons. In addition, it will examine the behavioral consequences of chronic exposure to these toxins.

SPECIFIC AIMS: 1) To expose Wistar-Kyoto (WKY) rats (stress-hyper responsive) and Sprague-Dawley (SD) rats (control) rats to chronic stress and then study the sensitivity to pyridostigmine bromide by measuring startle and neuroendocrine responses and the sensitivity to aromatic hydrocarbons by measuring the activity of hepatic enzymes induced to metabolize these compounds. 2) To treat WKY and SD rats chronically with these compounds and examine behavioral rhythms, temperature rhythms, startle responsiveness, and neuroendocrine function (both basally and in response to stress).

METHODOLOGY: SD and WKY rats will be treated with pyridostigmine bromide for 7 days and for the last 3 days will be subjected to a chronic stress regime. 24 hours after the last stressor exposure, the neuroendocrine and brain c-fos responses to pyridostigmine bromide will be evaluated, as well as the levels of specific isozymes of cytochrome P-450 in the liver. These enzymes are responsible for the metabolism of a large group of xenobiotics, including aromatic hydrocarbons. The changes in these enzyme levels will also be evaluated following stressor exposures and subsequent exposure to aromatic hydrocarbons. Similar studies will determine the behavioral and some physiological effects of chronic treatment with pyridostigmine bromide and aromatic hydrocarbons, as well as their effects on the responses to chronic stress.

EXPECTED PRODUCTS (MILESTONES): We expect that chronic stress will persistently increase the sensitivity to pyridostigmine bromide, which might explain how a drug that is used extensively in civilian populations might become toxic under the stresses of the battlefield. We also expect that this effect will be more pronounced in WKY rats, which would show that genetic predisposition might explain why some veterans developed unexplained illnesses and others did not. We expect that hepatic P-450 levels will be inhibited more in WKY rats, and that these results could explain a greater sensitivity to toxins metabolized by these enzymes. Finally, we expect that chronic exposure to pyridostigmine bromide or aromatic hydrocarbons might show long-term effects on behavior or body temperature rhythms which could be very sensitive indices of chronic toxicity.

STATUS/RESULTS TO DATE: We have been unable to validate the behavioral hyper responsiveness in WKY rats that has been reported by others. Preliminary data suggest that pyridostigmine bromide may cause a substantial decrease in voluntary running activity in rats, but it is unclear as yet whether this could be a long-term effect. However, it is clear that WKY rats are more sensitive to some effects of pyridostigmine bromide than SD rats. For example, WKY rats show an exaggerated startle response 1 to 3 weeks after ending pyridostigmine bromide treatment that is not present earlier and does not develop in SD rats. These results have led to the current studies of interactions among strain, chronic stress, and potential pyridostigmine bromide toxicity. This project was terminated in December 1997. Continuation of this project has been funded under a VA Merit Review grant to Dr. Richard Servatius, see Project # VA-49.

PUBLICATIONS: Servatius R. Ottenweller J. Beldowicz D. Guo W-D. Zhu G. Persistently

exaggerated startle responses in rats treated with pyridostigmine bromide. Journal of Pharmacology and Experimental Therapeutics, 287:1020-28, 1998.

Servatius R, Ottenweller J, Natelson B, Guo W-D, Beldowicz D, Zhu G. Interaction between inescapable stress and treatment with pyridostigmine bromide on plasma butyrylcholinesterase and the acoustic startle response in rats. Submitted to Journal of Pharmacology and Experimental Therapeutics (manuscript submitted).

Ottenweller J, Servatius R, Zhu GP, Beldowicz D, Natelson B. Persistent changes in physiology and behavior after pyridostigmine in rats with low BuChE. Conference on Federally Sponsored Gulf War Veterans' Illnesses Research, Wash., DC 1998.

Title: Core Program: Portland Environmental Hazards Research Center: Environment, Veterans Health and the Gulf War Syndrome. Core Project for Clinical and Epidemiology Research

Project #: VA-6Core

Agency: VA

Study Location: VAMC Portland

Project Status: Ongoing

Research Type: Epidemiology Research

P.I.: Peter Spencer, Ph.D.

Research Focus: Symptoms/General Health

Start Date (CY): 1994

Est. Completion (CY): 1999

OVERALL PROJECT OBJECTIVE: To illuminate the relationships between exposures (chemical, physical, stress) and unexplained illness in Gulf War veterans.

SPECIFIC AIMS: 1. To define 'caseness' of unexplained illness in Gulf War veterans; 2. To develop a questionnaire of exposures and effects based on the results of veteran interviews and other relevant data; 3. To conduct a case-control study focused on the spatial-temporal relationship between exposures and illness; 4. To provide subjects for research conducted in Projects I (6A) and II (6B).

METHODOLOGY: An epidemiological study using a population-based case-control design is being implemented. Pilot work was conducted in two phases before final determination of the research protocol. The first phase consisted of fourteen open-ended interviews with veterans with varying degrees of health and diverse military exposures. The interviews were completed in the Spring of '95. The second phase of the pilot work was a feasibility study in which a sample of 422 subjects, randomly chosen from a data tape of Oregon and Washington residents serving in the Persian Gulf, were mailed a survey describing the proposed plan for the large epidemiology study. Specifically, the researchers sought information on potential response rates and barriers to participation. Modifications of the planned research protocol were made based on responses of the feasibility study.

The regional study consisted of mailing a questionnaire seeking information on exposures in the Gulf and current health status to approximately 3000 northwest PGW veterans. The data base used for the sampling frame was obtained from the DoD (Defense Manpower Data Center). The database contains the names/addresses of military personnel who were deployed to the Persian Gulf from August 1990 through July 1991, and who listed Oregon or Washington as their home state-of-record. The accuracy of the addresses in the DoD database was determined in the feasibility study. Arrangements were made with the Department of Veterans Affairs, the National Institute for Occupational Safety and Health, and the Internal Revenue Service to track individuals with invalid addresses in the DoD file. In addition, we have utilized the services of Telematch, Equifax, Transunion and numerous Internet search engines to assist in finding current addresses and telephone numbers. The questionnaire is mailed in stages based on geographic location of the potential subjects. The survey methodology was developed with the assistance of consultants. Females and veterans in specific deployment groups (pre-combat, combat, post-combat) were over-sampled. From those who respond to the mailed questionnaire, potential cases and controls (total approximately 500) are recruited for the clinical case-control study. The

mailing of the survey questionnaire began in mid-November 1995 and the case-control clinical evaluations began in January 1996.

The working case definition determined at the conclusion of the May 1995 Research Retreat (with input from our External Advisory Committee) is as follows: Must have at least one of the following signs or symptoms to be defined as a potential case of Persian Gulf War Unexplained Illness (PGWUI). Onset must be during or after deployment to the Persian Gulf. Symptoms must have persisted for one month or longer and have occurred during the three-month period preceding the proposed physical examination.

- Muscle/joint pain;
- Cognitive changes including memory loss, confusion, inability to concentrate, mood swings, and/or somnolence;
- Diarrhea;
- Skin or mucous membrane lesions;
- Unexplained fatigue, plus at least four associated symptoms.

Potential cases and controls receive a medical workup at the Portland VAMC. Those participating in the clinical workup at the Portland VAMC also serve as the subjects in Projects I (VA-6A) and II (VA-6B). Multivariate analyses will be conducted to compare subjects with unexplained illness and healthy subjects with respect to reported and known exposures to specific environmental factors.

EXPECTED PRODUCTS (MILESTONES): Definition of associations, and the strength of those associations, between exposures and unexplained illness

STATUS/RESULTS TO DATE: 1. Open-ended interviews of PGW veterans have been completed, transcribed, and summarized. Information gleaned from the interviews was incorporated in the questionnaire content.

2. Feasibility study completed on 422 randomly selected Oregon veterans in the population to be studied. Changes in the design were implemented as a result of the findings:

- A. Compensation of \$10 is given to all subjects who complete and return the mailed survey questionnaire.
- B. The first year of survey mailing focused on residents of Oregon. Initial mailing was to subjects randomly selected within a 50-mile radius of Portland. Subsequent mailings were done to different geographic areas of the State of Oregon. The final survey sampling will be representative of the geographic distribution of all potential subjects in the data base obtained from the DoD.
- C. The mailing of questionnaires to potential subjects in areas outside of Portland, Oregon coincided with the negotiation of clinic space in VA facilities in outlying areas of the State. This change was needed because of the sizeable proportion of subjects in the feasibility study who reported that they would not participate in the planned study due to the traveling time to Portland VAMC.

3. Survey sampling plan for 1996-1998 is currently being implemented. Questionnaire returns are being scanned for data entry. Incomplete addresses are being tracked. As of December 1, 1997, 1982 questionnaires have been mailed to veterans who served in the PGW. Follow-up of the first two mailing waves (consisting of re-mailing questionnaires, telephone calls, and telephone-administered questionnaires) has been completed. A response rate of 58% has been achieved for the first six mailing waves. Follow-up for subsequent mailing waves is ongoing.

4. Clinical testing of subjects responding to mail survey began in January 1996. As of January, 1998, 409 subjects have been evaluated in the case-control study. Clinical testing will be completed in April 1998.

5. Mailings were made to 259 subjects living in rural areas of Oregon. In both April 1997 and June 1997, we completed four days of satellite clinical evaluation of 45 of the responders from this sample in cooperation with the Roseburg, Oregon, VAMC. In September and December 1997, we completed four

days of satellite clinical evaluations of 33 of the responders from the Washington sample in cooperation with the American Lake VAMC in Tacoma, Washington.

6. Serum samples from PGWUI cases and controls are studied with an antibody-capture ELISA test which is being developed for the detection of exposure to/infection with *Leishmania tropica*. An initial batch of 200+ serum samples from cases (two-thirds) and controls contained approximately 10% that had values more than three standard deviations from the mean of negative controls. While these samples fell within the range of values obtained from sera obtained from subjects with cutaneous *L. tropica* infection (positive controls), the specificity and sensitivity of this test-under-development has yet to be established (see VA-6E).

7. Analyses have been completed in the following areas:

Comparison of self-reported symptoms and symptoms reported at time of clinical evaluation;
Reliability of self-reported exposure information.

8. Analyses are in progress in the following areas:

Prevalence of self-reported symptoms in survey responders;
Prevalence of symptoms in veterans serving in specific deployment time periods;
Association between self-reported symptoms and clinical findings with time of deployment;
Association between self-reported symptoms/clinical findings and specific exposures that reportedly took place while in SW Asia.

PUBLICATIONS: Ludolph AC, Spencer PS. Toxic neuropathies and their treatment. In: Peripheral Neuropathies, Part 2, Balliere's Clinical Neurology; International Practice and Research (ed. Hartung HP), Balliere, London, 1996.

Spencer PS, Schaumburg HH, Ludolph AC. Experimental and Clinical Neurotoxicology, 2nd edition, Oxford, New York, in press

Title: Portland Environmental Hazards Research Center Program

Project #: VA-6

Agency: VA

Study Location: VAMC Portland

Project Status: Ongoing

P.I.: Bourdette, M.D./ Spencer, Ph.D.

This is the parent Program for VA projects VA-6A through VA-6E.

OVERALL PROJECT OBJECTIVE: To illuminate the relationships between exposures (chemical, physical, stress) and unexplained illness in Gulf War veterans.

SPECIFIC AIMS: 1. To define "caseness" of unexplained illness in Gulf War veterans; 2. To develop a questionnaire of exposures and effects based on the results of veteran interviews and other relevant data; 3. To conduct a case-control study focused on the spatial-temporal relationship between exposures and illness; and, 4. To provide subjects for research conducted in Projects I, II and part of IV.

METHODOLOGY: This is an epidemiological study using a population-based case-control design. Pilot work was conducted in two phases before final determination of the research protocol. The first phase consisted of fourteen open-ended interviews with veterans with varying degrees of health and diverse military exposures. The interviews were completed in the Spring of 1995. The second phase of the pilot work was a feasibility study in which a sample of 422 subjects, randomly chosen from a data tape of Oregon and Washington residents serving in the Persian Gulf, was mailed a survey describing the proposed plan for the regional epidemiology study. Information was sought on potential response rates and barriers to participation. Modifications of the planned research protocol were made based on responses in the feasibility study.

The design of the regional study consisted of mailing a questionnaire seeking information on exposures in the Gulf and current health status to approximately 3000 northwest PGW veterans in 1996-97. The data base used for the sampling frame was obtained from the DoD Defense

Manpower Data Center. The database contains the names/addresses of military personnel who were deployed to the Persian Gulf from August 1990 through July 1991, and who listed Oregon or Washington as their home state-of-record. The accuracy of the addresses in the DoD database was determined in the feasibility study. Arrangements were made with the Department of Veterans Affairs, NIOSH, and IRS to track individuals with invalid addresses in the DoD file. In addition, Telematch, Equifax, Transunion and numerous internet search machines were used to assist in finding current addresses and telephone numbers. The questionnaire was mailed in stages based on geographic location of potential subjects. The survey methodology was developed in consultation with Jim Bethel, Ph.D., Survey Statistician, Westat Inc. Females and veterans in specific deployment groups (pre-combat, combat, post-combat) were over-sampled. From those who responded to the mailed questionnaire, cases and controls (total approximately 500) were recruited for the clinical case-control study. The mailing of the survey questionnaire began in mid-November 1995 and the case-control clinical evaluations began in January 1996. The working case definition determined at the conclusion of the May 1995 Research Retreat (with input from an External Advisory Committee) was as follows: Must have at least one of the following signs or symptoms to be defined as a "Case". Onset must be during or after deployment to the Persian Gulf. Symptoms must have persisted for one month or long and have occurred during the three month period preceding the proposed physical examination.

- Muscle/joint pain;
- Cognitive changes including memory loss, confusion, inability to concentrate, mood swings, and/or somnolence;
- Diarrhea;
- Skin or mucous membrane lesions;
- Unexplained fatigue.

Healthy controls and cases were identified from responses to the mailed questionnaire. Cases and controls received a medical workup at the Portland VAMC. Cases and controls participating in the clinical workup at the Portland VAMC also served as the subjects in Project I and II. Multivariate analyses will be conducted to compare subjects with unexplained illness and healthy subjects with respect to reported and known exposures to specific environmental factors.

EXPECTED PRODUCTS (MILESTONES): Definition of associations, and the strength of those associations, between exposures and unexplained illness.

STATUS/RESULTS TO DATE: 1. Open-ended interviews of PGW veterans have been completed, transcribed, and summarized. Information gleaned from the interviews was incorporated in the questionnaire content.

2. Feasibility study has been completed on 422 randomly selected Oregon veterans in the population to be studied.

3. Survey sampling plan for 1996-1998 has been completed. As of August 1, 1998, we had received 1136 completed surveys (out of 2020 mailed surveys - 56% response rate).

4. Clinical testing of subjects responding to mail survey was completed in March 1998. 443 subjects were evaluated in the case-control study.

5. Mailings were made to 259 subjects living in rural areas of Oregon. In April 1997, and June 1997 we completed four days of satellite clinical evaluation of 45 of the responders from this sample in cooperation with the Roseburg, OR Veterans' Administration Medical Center. In September and December 1997, we completed four days of satellite clinical evaluations of 33 of the responders from the Washington sample in cooperation with the Great Lakes VAMC in Tacoma, Washington.

6. Analyses have been completed in the following areas: (1) Comparison of self-reported symptoms and symptoms reported at time of clinical evaluation; (2) Reliability of self-reported exposure information; and, (3) The association between deployment period and frequency of unexplained illness.

7. Analyses in progress: 1) Association between self-reported symptoms/clinical findings and specific exposures that took place while in SW Asia; 2) Description of the proportion of cases seen in the PEHRC clinic that fulfills the CDC 1988 and 1994 Chronic Fatigue definitions. Analysis of the characteristics of veterans with fatiguing symptoms. Description of the extent with which fatigue overlaps with fibromyalgia and multiple chemical sensitivity. Comparison of the neuropsychological and

neurobehavioral test results of cases with fatigue and other types of case symptoms; 3) Comparisons of objective clinical findings (i.e. physical examination, neurological findings, laboratory tests) between veterans presenting with different symptom types and healthy controls; 4) Description of the types of health complaints necessitating additional or supplemental medical referrals in our PEHRC clinic population. Compliance with recommended medical evaluation.

8. Manuscripts under review or in preparation based on study results on the following topics:

- Subjective and objective evidence of unexplained illness in veterans of the Persian Gulf War;
- Validation of self-reported symptoms in PGW veterans by clinical evaluation
- Strategies to assess validity of self-reported exposures during the PGW
- Chronic Fatigue in a Population of Veterans Deployed to the Gulf War

9. Findings to Date: 1) The utility of our working case-definition was assessed and changes made. The definition was modified to reduce the number of symptom categories from five to three (unexplained fatigue, musculoskeletal complaints, and cognitive/psychological complaints). 2) The reliability and validity of self-reported exposure data were assessed and reported in manuscripts and presentations by McCauley and Spencer, 1997 and 1998. Timeline - Completion of data analysis and reports is anticipated by September 1999.

PUBLICATIONS: Spencer PS, McCauley LA, Joos SK, Lasarev MR, Shuell T, Bourdette DN, Barkhuizen A, Johnston W, Storzbach D, Wynn M, Grewenow R, Other Members of the Portland Environmental Hazards Research Center. U.S. Gulf War veterans: Service periods in theater, differential exposures and persistent unexplained illness. *Toxicology Letters*. (In press).

McCauley LA, Joos SK, Lasarev MR, Storzbach D, Bourdette DN, Other Members of the Portland Environmental Hazards Research Center. (submitted) Persian Gulf War unexplained illness: Persistence and unexplained nature of self-reported symptoms.

McCauley LA, Joos SK, Spencer PS, Lasarev MR, Shuell T. Other Members of the Portland Environmental Hazards Research Center. (Submitted). Strategies to assess validity of self-reported exposures during the Persian Gulf War.

Gold BG, Schnell J, Spencer PS. Neurotoxic Disorders. In: Rosenberg RN, ed, *Atlas of Clinical Neurology*, Butterworth-Heinemann, Philadelphia, 1998, pp. 14.1-14.22.

Spencer PS, Wilson B, Albuquerque EX. Sarin, other "nerve agents" and their antidotes. In: Spencer PS, Schaumburg HH, eds, *Experimental and Clinical Neurotoxicology*, New York: Oxford University Press; 1999. (In press).

Frank AF, Blythe LL, Spencer PS. Aspects of veterinary neurotoxicology. In: Spencer PS, Schaumburg HH, eds, *Experimental and Clinical Neurotoxicology*, Oxford University Press, New York; 1999, (In press).

Spencer PS. N,N-Diethyl-m-toluamide. In: Spencer PS, Schaumburg HH, eds, *Experimental and Clinical Neurotoxicology*. New York: Oxford University Press; 1999. (In press).

Spencer PS. Biological principles of chemical neurotoxicity. In: Spencer PS, Schaumburg HH, eds, *Experimental and Clinical Neurotoxicology*, New York: Oxford University Press; 1999. (In press)

Spencer PS. Diethylbenzene. In: Spencer PS, Schaumburg HH, eds, *Experimental and Clinical Neurotoxicology*, New York: Oxford University Press; 1999. (In press).

Spencer PS, Schaumburg HH. *Experimental and Clinical Neurotoxicology*, 2nd edition, New York: Oxford University Press; 1999. (In press) (Book).

Title: Psychosocial, Neuropsychological and Neurobehavioral Assessment (Project I)

Project #: VA-6A

Agency: VA

Study Location: VAMC Portland

Project Status: Ongoing

Research Type: Clinical Research

P.I.: Kent Anger, Ph.D.

Research Focus: Brain & Nervous System, Symptoms/General Health

Start Date (CY): 1994

Est. Completion (CY): 1999

OVERALL PROJECT OBJECTIVE: To determine if veterans have developed adverse neurobehavioral or psychological effects from service in the Persian Gulf War. If so, to determine the triggering events, including combat stressors, life stressors, chemical exposures, personality disorders, psychological health and motivation to seek compensation

SPECIFIC AIMS: 1. Determine if there are objective memory and attention deficits in Veterans reporting (unexplained) symptoms which developed during or since the Gulf War; 2. Determine the relationships between neurobehavioral and psychological measures and unexplained symptoms referable to the Gulf War; 3. Employ in-depth neuropsychological examinations to identify neurobehavioral, neuropsychological and psychosocial factors that may explain or more fully characterize cognitive symptoms or objective cognitive deficits.

METHODOLOGY: This project will determine if veterans of the 1991 Persian Gulf conflict have developed any of a broad range of adverse neurobehavioral or psychological health effects attributable to triggering events during their service in the 1991 Gulf War. Hypothesized triggering events include combat or life stressors and chemical exposures. Potential factors that could contribute to or influence the presence of adverse health effects will be assessed and entered into the analysis, including personality disorders, psychological health, medical conditions, and motivation to seek compensation.

Each veteran participating in the overall research program receives a screening assessment with a 4-hour battery of 18 standard, reliable and valid psychosocial, neuropsychological, and behavioral performance tests. The psychosocial and neuropsychological tests selected for the screening battery have been implemented in a user-friendly and consistent computer-administration format (Health Screening System) that does not require individual explanation for each test developed for this project. The neurobehavioral tests of attention, memory and coordination are implemented in the Behavioral Analysis and Research System (BARS), and two new tests (ODTP and Serial Digit Learning) were added to BARS for this project (Specific Aims 1 and 2).

During the course of the study, up to 50 "Cases" and 50 "Controls" (from VA-6) or referents will receive an in-depth clinical (neuropsychological) interview to more fully characterize possible disorders (Specific Aim 3). In addition, those initially demonstrating a high level of PTSD will be invited back after 1-2 years to repeat the tests to assess PTSD progression, resources permitting. Results are being analyzed, during data collection: (a) by experienced clinicians (neuropsychological exam; Specific Aim 3), (b) with multivariate techniques adjusted for confounders (neurobehavioral tests; Specific Aim 1), and (c) with principal components analysis, logistic hierarchical multiple regression, discriminant function analysis, latent growth models analysis, and structural equation modeling to characterize the nature of the psychosocial results (Specific Aim 2).

EXPECTED PRODUCTS (MILESTONES): Develop a predictive model relating the occurrence of unexplained illness to chemical exposure, psychosocial, or neuropsychological factors.

STATUS/RESULTS TO DATE: Methods Developed or Modified for Project Twelve valid, reliable psychosocial and neuropsychological tests were selected for the screening battery in this project. They address the main psychological factors identified: PTSD EVALUATION - Penn Inventory for PTSD; Mississippi PTSD Scale. PTSD Checklist. PSYCHOSOCIAL FACTORS - Beck Depression Inventory (BDI); Beck Anxiety Inventory (BAI); Substance Abuse Subtle Screen Inventory (SASSI); Life Experience Scale (LES). PERSONALITY VULNERABILITY - Positive Affect Negative Affect Scale (PANAS); Minnesota Multiphasic Personality Inventory-II (MMPI-II). HEALTH SYMPTOMS - Health Status Questionnaire (SF-36); Hopkins Symptom Checklist (SCL-90R). WAR ZONE EXPERIENCE - Combat Exposure Scale, revised (CES-r). A common presentation format was developed for the 12 diverse psychosocial and neuropsychological tests in order to improve acceptability and speed completion, using Supercard software for presentation on a Macintosh laptop computer. The new format includes a spoken test administration option (individually selectable and deselectable at any point in testing) which has

been digitally recorded for inclusion with the test presentation system and options to navigate within a test to any question or to repeat the instructions. A training program was also developed to teach veterans to complete the questionnaires, and durable external "DataSled" Response Units (developed in a separately-funded project) have been connected to the laptop computers on which the tests are being presented to up to 9 veterans simultaneously (number limited by space).

The new testing format (HSS) was employed with 20 PGW veterans and 10 civilians in pilot evaluations of the prototype training program. Test subjects were very positive about the testing system, and the mean questionnaire administration time approximated the target of 3 hours in a part of that group (Kovera et al., 1996). Changes recommended by respondents were introduced into the HSS. These tests are now being administered to all veterans tested by the PEHRC Epidemiology/Medical Core unit.

The neurobehavioral tests employed in this project were selected from the Behavioral Research and Assessment System (BARS): ATTENTION, MEMORY, and LEARNING - Symbol Digit, Digit Span (forward and backward), Selective Attention Test Serial Digit Learning; RESPONSE SPEED AND COORDINATION - Simple Reaction Time; MOTIVATION -Oregon Dual Task Procedure (ODTP) - (modeled after the Portland Digit Recognition Test [PDRT]). Most BARS tests were implemented under other funding to one of the project PIs (Anger), but the ODTP was prototyped and administered to pilot subjects for this project, including veterans involved in the psychosocial test development (above). The duration of testing met the target time of 60 minutes. These tests are now being administered to all Veterans tested by the PEHRC Epidemiology/Medical Core unit.

Reliability of Methods: During Summer, 1996, the 18 HSS and BARS tests used in this project were administered twice to 60 Oregonians, with one week between administrations, to evaluate their test-retest reliability. In general, reliability scores were comparable to those reported for the tests in their original format. This supports the use of the tests in the new HSS and BARS format which allows testing of larger numbers of veterans due to the increased cost-effectiveness of this system. A manuscript describing these results has been submitted for publication.

Website Describing HSS and BARS: In order to make the information about the methods used in this (and other) projects more widely available, a website has been created that describes the tests, presents test-retest reliability data, provides a simulation of training for the HSS, and depicts one screen for each test. Home Pages were thus created for both BARS and HSS. Both Home Pages are accessed through <http://home.att.net/~angerk>.

Results of Case-Control Comparisons: HSS and BARS tests have been administered to ~450 veterans, as part of the PEHRC clinical study (Specific Aims 1-2). The data reveal consistent and highly significant differences between cases and controls on most psychological test scales in the direction of increased distress in cases (veterans with unexplained symptoms) and slowed response latencies on several neurobehavioral tests in cases compared to controls. In addition, a subgroup has been identified that has very slow response latencies on a test of memory and attention. This subgroup is almost entirely comprised of cases (Anger et al., in press).

In-depth neuropsychological examinations (Specific Aim 3) have been administered to approximately 50 people by Drs. Storzbach and Binder. These tests require scheduling a full day in the event that a diagnostic interview is required based on initial testing. This has slowed the process when last-minute schedule changes, cancellations, or no-shows effectively lose the one subject that can be scheduled for each week. Recruitment has been difficult to accomplish, although aggressive contacts have increased the rate in the past six months.

PUBLICATIONS: Anger WK, Storzbach D, Binder LM, Campbell KA, Rohlman DS, McCauley L, Kovera CA, Davis KL. Neurobehavioral Deficits in Persian Gulf Veterans: Evidence from a population-based study. *Journal of the Internat Neuropsych Soc*, (In press).

Campbell KA, Rohlman DS, Storzbach D, Binder LM, Anger, WK, Kovera CA, Davis K, Grossmann S. Test-retest reliability of psychological and neurobehavioral tests self-administered by computer. *Assessment*. (In press).

Anger WK, Rohlman DS, Storzbach D. Neurobehavioral Testing in Humans. *Current Protocols in Toxicology* (M Maines, L Costa, IG Sipes, S Sasse, DJ Reed, eds). John Wiley & Sons.

New York. (In press).

Anger WK, Storzbach D, Amler RW, Sizemore OJ. Human behavioral neurotoxicology: Workplace and community assessments. In: Environmental and Occupational Medicine (third edition) (W Rom, Ed.) Little, Brown, and Co.(In press).

inder LM, Storzbach D, Anger WK, Campbell KA, Rohlman DS, & members of the Portland Environmental Hazards Research Center. Subjective cognitive complaints, affective distress, and objective cognitive performance in Persian Gulf War veterans. Archives of Clinical Neuropsychology. (In press).

Title: Clinical and Neuroendocrine Aspects of Fibromyalgia (Project II)

Project #: VA-6B

Agency: VA

Study Location: VAMC Portland

Project Status: Ongoing

Research Type: Clinical Research

P.I.: Robert Bennett, M.D.

Research Focus: Immune Function, Symptoms/General Health

Start Date (CY): 1994

Est. Completion (CY): 1999

OVERALL PROJECT OBJECTIVE: Determine the relationship between exposures during the Persian Gulf War and the development of fibromyalgia.

SPECIFIC AIMS: 1. Screen veterans complaining of fatigue and musculoskeletal symptoms with previously developed and validated questionnaires and directed physical examinations to determine if fibromyalgia is present. 2. Correlate the presence or absence of fibromyalgia with possible environmental exposures, as determined in the core part of this program, or with neuropsychological factors, as determined in Project I: Psychosocial, Neuropsychological and Neurobehavioral Assessment. 3. Evaluate a smaller group of veterans with fibromyalgia and an age and sex matched group of veterans without fibromyalgia, for the neuroendocrine abnormalities described in fibromyalgia.

METHODOLOGY: Utilizing the cases determined in the Portland case-control epidemiological study (see VA-6), using validated methods, screen cases with musculoskeletal complaints for fibromyalgia and controls without symptoms. Perform neuroendocrine testing on both cases and controls.

EXPECTED PRODUCTS (MILESTONES): 1. Association between exposure to the Persian Gulf War And the development of fibromyalgia. 2. Prevalence of neuroendocrine findings in a primarily male sample.

STATUS/RESULTS TO DATE: Preliminary work consisted of reviewing the Portland VA Persian Gulf War (PGW) Registry for cases of musculoskeletal pain. The prevalence of such complaints in the Portland PGW Registry sample was 29.6% compared to the National Registry prevalence of 13.9% and the DoD Comprehensive Clinical Evaluation Protocol reported prevalence of 47%. Results from a pilot population-based survey of 157 veterans eligible for our epidemiologic case-control study (see Project VA-6) indicated that the self-reported rates of musculoskeletal pain in both female and male subjects were both 38%. Responders to the mailed survey who had registered in the PGW registry were two times more likely to report musculoskeletal pain than responders who had not registered.

Investigators worked closely with the epidemiological core in the development of the items to include in the survey questionnaire, telephone screening questions, and the history and physical examination to be done in the case-control study. Exclusionary diagnoses were determined. The history and physical examination was pretested on three subjects with musculoskeletal pain in January 1996.

Since commencing the Persian Gulf Research Clinic in January 1996, the majority of subjects has been evaluated by a single examiner for rheumatological conditions. A blinded tender-point examination is performed followed by a full rheumatology history and physical examination. The Core laboratory data, which include an erythrocyte sedimentation rate, C-reactive protein,

antinuclear antibody, rheumatoid factor, hepatitis B, C, and human immunodeficiency virus serologies, and selected joint radiographs, are reviewed at the bimonthly Clinical Case Review meeting to arrive at a final rheumatological diagnosis.

To date we have analyzed the rheumatologic and neuropsychologic features of 349 deployed Gulf War veterans (32.8±8yrs;83%M) from the population-based case control study. Data are presented on 229 cases (unexplained musculoskeletal (MSS), cognitive/psychologic, fatigue, gastrointestinal and/or dermatologic illnesses) and 120 controls. Unexplained MSS pain was present in 84 cases (37%). Fibromyalgia was present in 50 and fatigue in 47 of the MSS cases. Cases were more likely than controls to have musculoskeletal complaints (86%vs27%; $p<0.001$). Some regional syndromes were more common amongst cases: mechanical backache (39%vs18%; $p<0.001$), myofascial pain syndrome (24%vs11%; $p=0.006$), and patellofemoral syndrome (12%vs2%; $p=0.002$). There was no difference for tendinitis (16%vs8%), osteoarthritis (8%vs4%), overuse syndrome (4%vs2%), or hypermobility (6%vs6%). Inflammatory conditions were infrequent (spondyloarthropathy 4, and SLE and RA 1 each). Elevated creatinine kinase levels were present in 15% of cases and controls; and ANA >1:40 in 2% of cases and 3% controls. MSS cases were significantly more likely to complain of muscle weakness or cramps, post-exertional pain, restless legs, headaches, chest pain, abdominal cramps, cold intolerance, tender skin, dry eyes; and sleep complaints of unrefreshed sleep or bruxism. MSS cases scored significantly higher on the Fibromyalgia Impact Questionnaire; Beck Anxiety and Depression Inventory; SF 36 (pain, vitality, physical and physical role function, health change, emotional role function, health perception and social function); and "somatization" sub-scales of the MMPI and SCL-90. MSS cases reported significantly more combat exposure and higher post traumatic stress scores (MISS, PENN, PCL C); with greater negative control and negative impact on the Life Events Scale. Finally, MSS cases and in particular those with fibromyalgia displayed neurobehavioral abnormalities of attention and memory (digit span backward, reaction time and Oregon Digit Recognition).

The second component entails study of the neuroendocrine axis of the Persian Gulf veterans. All subjects attending the Persian Gulf Research Clinic are screened using the serum Insulin-like Growth Factor-I (IGF-1) and Insulin-like Growth Factor Binding Protein 3 (IGFBP3) levels. This has previously been shown to be reduced in one-third of patients with fibromyalgia. In the current study, serum IGF-1 levels were not significantly different in subjects with unexplained muscle and joint pain (175.9±43.3), fibromyalgia (183.8 ng/ml±61.0), unexplained fatigue (193.4±59.9), or healthy controls i.e. (192.2±60.6).

The final component involves evaluating changes in the neuroendocrine axis in greater detail. We aim to study 20 cases of fibromyalgia with low IGF-1 levels and 20 control subjects with normal IGF-1 levels over the next 2 years for an in-depth study of their hypothalamic-pituitary-growth hormone and hypothalamic-pituitary-adrenal axis. The study protocol has been approved by a peer review committee of the Clinical Research Center, a dedicated research facility at the Oregon Health Sciences University, which is funded and regularly reviewed by scientists and clinicians from the National Institutes of Health. The laboratories of the Clinical Research Center and Oregon Graduate Institute perform half-hourly cortisol and growth hormone levels, a 24-hour cortisol production rate and two growth hormone stimulation tests. This will enable the research team to objectively document an altered neuroendocrine stress response and adult growth hormone deficiency in the symptomatic veterans. When testing on all subjects is completed, the altered neuroendocrine stress response will be correlated with the neuropsychological profile and, particularly, with the stress scores collected by Project I. Finally, the stress response will be correlated with reported exposures to various environmental agents and the deployment periods of the veterans (see Project VA-6).

To date intensive neuroendocrine axis testing have been completed in 11 subjects with FM (36±7.7yrs) and low IGF-1 levels (129±27ng/ml) compared to 8 controls (35±10yrs) with normal IGF-1 levels (240±26ng/ml). Using clonidine and L-arginine, 7/11 FM and 4/8 controls failed to increase their serum GH levels to >5ng/ml confirming the presence of adult growth hormone deficiency. In this small sample the mean peak GH achieved was not different between cases and controls (4.1±4.6ng/ml vs 3.5±3.4ng/ml). GH is normally secreted during stage 4 sleep in the mid to latter part of sleep. Studies of 24 hour serum GH secretion have demonstrated abnormal

patterns of secretion in civilians with FM. In the current study, FM cases have similar 24 hour GH levels but higher median levels from 8 pm to 2 am compared to controls (0.16ng/ml vs 0.05ng/ml; $p < 0.001$), suggesting a phase advance in GH secretion.

Future reports will provide more detailed diurnal data utilizing the more complicated method of deconvolutional analysis. The hypothalamic-pituitary-adrenal axis is another candidate for studies of the effects of stress. We found no difference in urinary cortisol between cases and controls (22.7+9.9ug/g vs 17.7+8.3ug/g creatinine). Utilizing the deuterated cortisol method there was no difference in 24 hour total serum cortisol production rate between cases and controls (17.3+7.7 vs 19+3.8 mg/24 hrs). The diurnal pattern of cortisol secretion also appears to be maintained with peak levels secreted in the early morning and nadir occurring in the early evening. Interestingly FM cases reached a significantly lower nadir than controls from 8 pm to 2 am (1.5ug/dl vs 1.9ug/dl; $p = 0.005$) at the same time when their GH levels were greater.

PUBLICATIONS: Bennett RM, Clark SR, Walczyk J. A randomized double blind placebo-controlled study of growth hormone in the treatment of fibromyalgia. *Amer J Med*, 104:227-34, 1998.

Barkhuizen A, Rosen H, Wolf S, Flora K, Benner K, Bennett R, Musculoskeletal pain and fatigue is associated with chronic hepatitis C: a report of 239 hepatology clinic patients. *Am J Gastroenterol*. (In press).

Barkhuizen A, Campbell S, Bennett R, Storzbach D, Lazarev M, Rohlman D, Anger K, Binder L, Campbell K, and the PEHRC team. Musculoskeletal syndromes, neuropsychological features and IGF-1 levels of deployed Persian Gulf War veterans. *Arthritis Rheum* 40:S214 1998 (Abstract Discussion session at 1998 ACR National Meeting).

Title: Neurotoxicity of Environmental Pollutants and Warfare Agents (Project III)

Project #: VA-6C

Agency: VA

Study Location: VAMC Portland

Project Status: Ongoing

Research Type: Mechanistic

P.I.: Rosemarie Drake-Baumann, Ph.D.

Research Focus: Pyridostigmine Bromide, Environmental Toxicology

Start Date (CY): 1994

Est. Completion (CY): 1999

OVERALL PROJECT OBJECTIVE: To evaluate the neurotoxic potential of a group of hydrocarbon solvents and the effects on cholinergic neurons of long-term exposure to pyridostigmine bromide (PB).

SPECIFIC AIMS: 1. To evaluate effects of pyridostigmine bromide on cholinergic function in cerebellar and spinal cord explants. 2. To examine the relationship between the chromogenic and neurotoxic properties of selected organic solvents.

METHODOLOGY: Spinal cord-muscle cocultures derived from fetal mice are used to study to effects of chronic and acute exposure to pyridostigmine bromide on the neuromuscular junction. Cerebellar cultures derived from newborn mice are used to study the relationship between chromogenic and neurotoxic properties of a group of monocyclic and dicyclic hydrocarbon solvents. Test substances are incorporated into the culture medium. The dosage and duration of exposure to pyridostigmine and each of the solvents are experimentally determined. Morphological Analyses - Control and treated explant cultures are stained with thionin (for Nissl substance). This stain allows the evaluation of the various neuronal cell groups present in explant cultures by light microscopy. Central and peripheral myelination can be readily examined in the living state using bright-field optics. In fixed preparations, peripheral myelination can be examined using Sudan black staining. Histochemical staining for acetylcholinesterase is used for localization of neuromuscular junctions in muscle fibers. Immunohistochemical staining is used to identify alterations of specific neural components (neurofilaments, calbindin), neurotransmitters (choline acetyltransferase, acetylcholinesterase) and supportive glial cells (glial fibrillary acidic protein) induced by neurotoxic compounds. Synaptic changes are elucidated by electron

microscopy.

Electrophysiology - In spinal cord-DRG-muscle explants, extracellular electrodes are used to record dorsal horn, ventral horn and muscle responses evoked by extracellular stimulation of dorsal and ventral roots. In cerebellar cultures, neurons develop spontaneous activity as they mature in vitro. Extracellular recordings are used to obtain single unit discharge rates and, in combination with extracellular stimulation, are used to assess the efficacy of excitatory and inhibitory synaptic circuitry. Intracellular electrodes are used to study the active and passive properties of individual neurons (motoneurons in the spinal cord cultures and Purkinje cells in cerebellar cultures) and their synaptic responses to afferent stimulation and to record muscle activity.

EXPECTED PRODUCTS (MILESTONES): Electrophysiological and morphological characterization of the cellular neurotoxicity of selected hydrocarbon solvents and pyridostigmine bromide.

STATUS/RESULTS TO DATE: Pyridostigmine Bromide: The effects of extended exposure (1-2 weeks) to low concentrations of PB (comparable to those associated with doses taken by Operation Desert Storm troops) on neuromuscular function were examined in a well characterized in vitro model. Organotypic spinal cord-DRG explants co-cultured with skeletal muscle were used to evaluate the effects of exposure to PB. These combination cultures reproduce the ultrastructure, physiology and pharmacology of comparable tissues in the intact animal, and they respond pathophysiologically and neuropathologically to chemicals with neurotoxic properties in a comparable manner.

No physiological or ultrastructural alterations were observed in DRG neurons or ventral horn motoneurons with exposure to 10^{-6} M PB for periods of up to two weeks in vitro. Neuromuscular function was selectively affected by PB treatment. Acute treatment (minutes) with PB increased spontaneous muscle contraction. This potentiation is consistent with the inhibition of acetylcholinesterase and the consequent greater availability of acetylcholine at the neuromuscular junction. This effect could be blocked by succinylcholine. Long-term treatment (days to weeks) with PB decreased the contractile activity of muscle fibers and their sensitivity to externally applied acetylcholine. Electron microscopical examination of the neuromuscular junctions in cultures treated with PB revealed no evident structural changes after a 1-week exposure but, after a two-week treatment, cystic degenerative changes were evident in the nerve terminals. The postjunctional folds displayed atrophic changes and were surrounded by vacuolar spaces. These changes are indicative of both nerve terminal degeneration and a subjunctional myopathy. Comparable changes have been reported in mammals treated with anticholinesterases. Within one week following withdrawal of PB, muscle fibers became functional indicating that the neuromuscular effects of PB were reversible. This study indicates that the use of low-dose PB may not be entirely risk-free. Even at low concentrations, exposure to PB for 1 or 2 weeks produced early functional and later structural alterations of the neuromuscular junction. In early stages, changes were limited to desensitization of the acetylcholine receptors produced by the persistence of acetylcholine in the synaptic cleft, followed in later stages by degenerative changes of the neuromuscular junction. Comparable changes in animals or humans would likely be associated with muscle weakness. However, the reversibility of the PB induced neuromuscular effects suggests that the symptom of fatigue which persists in Gulf War veterans are causally unrelated to the phenomenon observed in spinal cord-muscle cultures. **Bromide Anion:** PB is composed of two moieties, pyridostigmine (anticholinesterase) and bromine. Bromine substitutes for chlorine and, in large concentrations, may generate toxic effects expressed in the form of dermatological and neurological changes. The bromide anion permeates chloride channels of cell membranes and in contrast to pyridostigmine; it readily diffuses across the blood-brain barrier. The aim of this study was to examine the effect of bromide on neural activity. Cerebellar explant cultures were exposed for a period of two weeks to 10^{-6} M of sodium bromide. Neural activity was subsequently evaluated by electrophysiological recordings. Exposure to micromolar concentrations of bromide had no effect on the spontaneous activity of cerebellar neurons. Additional studies showed that a significant depression in the cerebellar neural activity resulted only after substitution of sodium chloride in the nutrient media with much higher concentration (20 mM) of sodium bromide. At this concentration, bromide has been shown to enhance gamma-

aminobutyric acid (GABA)-activated currents in cultured cerebral neurons. The depressant effects elevated bromide on neural activity were reversed by incubation with standard saline solution. These results suggest that the concentration of the bromide anion in PB at low doses (comparable to doses used by Operation Desert Storm troops) produces negligible effects on neural function.

During FY 98 we initiated experiments to establish whether exposure of murine spinal cord muscle co-cultures to certain organophosphates (e.g. diisopropyl fluorophosphate, DFP), besides exerting a cholinergic effect on the neuromuscular junction, could also induce the delayed degeneration of myelinated nerve fibers (OPIDN) seen in animals several weeks after exposure, and to test whether pretreatment with pyridostigmine can prevent the development of OPIDN. Cultures were exposed to 100 µg/ml of DFP for 1, 2 and 3 successive 24 hr intervals. A single 24 hr. exposure did not produce delayed morphological alterations of central and peripheral axons. Peripheral abnormalities developed in myelinated fibers within two weeks following multiple exposures.

During FY 98 we began examine the neurotoxic properties of representative examples of the class of aromatic hydrocarbon solvents. The compounds we are evaluating at present are two isomers of diethylbenzene with different chromogenic properties and neurotoxic potential in animals. Animal data suggest the two phenomena are mechanistically linked. Diacetylbenzene, the reported active metabolite of diethylbenzene is both chromogenic and neurotoxic to neuronal cultures. Exposure to diacetylbenzene even at low concentrations induced peripheral alterations of myelinated fibers and also affected the electrophysiological properties of neurons. Ongoing studies are aimed examining the specificity of these effects. These two projects should be completed by the end of fiscal year 1999.

PUBLICATIONS: Drake-Baumann R, Seil FJ, Spencer PS. Neuromuscular responses to pyridostigmine bromide in organotypic spinal cord-muscle culture. J Physiol. (Paris), 92:305-308, 1998.

Drake-Baumann R, Seil FJ. Effects of long-term exposure to pyridostigmine on neuromuscular junctions in vitro. (Submitted).

Title: DNA Damage from Chemical Agents and Its Repair (Project IV)

Project #: VA-6D

Agency: VA

Study Location: VAMC Portland

Project Status: Ongoing

Research Type: Mechanistic

P.I.: Glen Kisby, Ph.D.

Research Focus: Chemical Weapons, Diagnosis

Start Date (CY): 1994

Est. Completion (CY): 1999

OVERALL PROJECT OBJECTIVE: Determine the long-term consequences of genotoxin exposure on nervous system and non-nervous system tissue.

SPECIFIC AIMS: 1. Determine the type and quantity of specific DNA adducts and the capacity to repair these adducts in control and nitrogen mustard (HN2) treated genomic DNA isolated from normal human skin and brain; 2. Determine the type and quantity of specific DNA adducts and the capacity to repair these adducts in genomic DNA isolated from mouse cerebral cortex cultures treated with HN2; 3. Assess the relationship between DNA damage, DNA repair and cell degeneration in rodent neuronal cultures treated with HN2; 4. Compare and contrast DNA damage, DNA repair and cytotoxicity in primary neuronal and primary glial cultures treated with HN2.

METHODOLOGY: 1. Determine the relative sensitivity of different nervous tissue cell types to HN2. Nervous tissue cell cultures (e.g., rodent astrocytes, cerebellar granule cells, cortical explants, and SY5Y human neuroblastoma cells) will be treated for various time periods with a range of HN2 concentrations and examined for cytotoxicity by using fluorescent dyes for live

(e.g., the mitochondrial fluorochrome Rhodamine 123) and dead (e.g., the nuclear DNA binding fluorochrome propidium iodide) cells. 2. Develop sensitive methods for the detection of DNA adducts (i.e., monofunctional and cross-links) in HN2 treated nervous tissue and human skin explants. The most sensitive methods for detecting HN2-induced DNA adducts are the 32P-postlabeling/TLC (monofunctional adducts) and ethidium bromide assay (crosslinks). Therefore, the 32P-postlabeling/TLC and ethidium bromide assay method was developed and used to detect DNA adducts in tissue treated for periods up to 7 days with HN2. 3. Develop methods for determining DNA repair levels and activity in mustard treated neuronal (e.g., cortical, cerebellar) and non-neuronal (e.g., astrocytes) cultures and human skin. The predominant DNA adduct produced by sulfur and nitrogen mustards is the alkylated guanine DNA adduct. The alkylated guanine DNA adduct is repaired by the base-excision repair (BER) pathway. The rate-limiting step in BER is the repair of an apurinic site by apurinic/apyrimidinic endonuclease (APE). APE levels and activity will be determined in nervous tissue treated with HN2. APE levels will be determined by western blotting and activity will be determined using damaged plasmid DNA. These studies will determine the relative DNA repair of HN2-induced DNA adducts in different CNS cell types. Findings from these studies will be important for establishing a possible relationship between DNA damage, genotoxin exposure and cytotoxicity of nervous tissue.

EXPECTED PRODUCTS (MILESTONES): 1. Determination of whether mustard agents can induce persistent DNA damage to nervous tissue.
2. Development of sensitive techniques for the detection and quantitation of DNA adducts induced by mustards.

STATUS/RESULTS TO DATE: 1. We began study of the response of neuronal and glial cells and human skin tissue to HN2. Human and rodent postmitotic neurons proved to be more sensitive to HN2 than glial cells.
2. We examined the acute (24 h) and long-term (7 day) cytotoxic effect of HN2 on human skin tissue (SKIN2tm). HN2 is acutely toxic to human skin tissue at high concentrations but extensive tissue damage can be induced by lower concentrations (1/10 or 1/100 of the acute dose) when tissue is exposed for long periods of time.
3. We developed assays to measure the predominant HN2-induced DNA adduct (monofunctional; GN7) and cross-links. We demonstrated that guanine-N7(GN7) levels were 2-fold higher in postmitotic rat cerebellar neurons than comparably treated dividing SY5Y cells. We showed that cross-link levels in HN2-treated human SY5Y neuroblastoma cells increased with increasing concentrations of HN2. Studies are underway to determine if these adducts persist in HN2-treated nervous tissue.
4. We developed an assay for examining the relationship between cytotoxicity and DNA damage.
5. We developed assays for measuring the effect of HN2 on neuronal and human skin tissue DNA-repair levels and activity.
6. Nitrogen mustard (HN2) modulated the level and activity of nervous tissue DNA-repair protein APE, which specifically repairs the monofunctional HN2 DNA adduct. Apurinic apyrimidinic (APE) levels increased in HN2-treated rodent neuronal cultures (non-dividing cells) while APE levels decreased in human neuronal cultures (dividing cells). APE activity increased in HN2-treated human skin cultures (actively dividing cells). Altered levels and activity of APE may be indication of HN2-induced DNA damage.
7. Nitrogen mustard (HN2) modulated the level and activity of APE in human skin tissue. A significant increase in immunostaining was demonstrated in human skin probed with an antibody to APE. In support, APE activity was increased in cultured human skin explants treated with HN2.
8. Lymphocytes from a small group of psychologically stressed PGW veterans were examined for DNA repair (i.e., APE levels and activity). APE levels and activity were increased in lymphocytes of subjects exhibiting psychological stress with accompanying somatic symptoms. Recent studies explored the effect of repetitive stress on brain tissue DNA repair. Coronal brain tissue sections of stressed and unstressed mice were immunoprobed with an antibody to APE (previously used to determine human lymphocyte APE levels) and a significant increase in immunostaining was observed in the hippocampus of stressed mice when compared with unstressed mice. These studies suggest that stress (e.g., psychological) may perturb brain tissue DNA repair.

PUBLICATIONS: Kisby GE, Springer N, Spencer PS. In Vitro neurotoxic and DNA-damaging properties of nitrogen mustard (HN2). (In press).
Spencer PS, Daniels J, Kisby GE. Mustard warfare agents and related substances. In: Spencer PS, Schaumburg HH, eds, Experimental and Clinical Neurotoxicology. New York: Oxford University Press; 1999. (In press).
Kisby GE, Sweatt, Spencer PS. Role of DNA repair in protecting mature nervous tissue from DNA damage. J Cell Biochem 21A, 348, 1995.

Title: Clinical and Epidemiology Leishmania Research

Project #: VA-6E

Agency: VA

Study Location: VAMC Portland

Project Status: Ongoing

Research Type: Clinical Research

P.I.: Michael Riscoe, Ph.D.

Research Focus: Leishmaniasis, Diagnosis, Treatment

Start Date (CY): 1994

Est. Completion (CY): 1999

OVERALL PROJECT OBJECTIVE: Development of serological test for viscerotropic leishmaniasis and determination of infection rate.

SPECIFIC AIMS: Core Objective: To determine the possibility and rate of infection of PGW veterans with Leishmania spp, and to develop novel anti-leishmanial chemotherapeutics.

METHODOLOGY: Immunological screening of patient and volunteer blood. All subjects participating in the case/control study have immunological screening of their blood for evidence of L. tropica infection (studies performed by Corixa Corp., Seattle, WA). Other pertinent topics being explored in the Experimental Chemotherapy laboratory of the Portland VAMC:

1. Novel chemotherapeutic agents for viscerotropic leishmaniasis.
2. Development of adjunctive therapy for use with existing medicinals used for treatment of Leishmaniasis, as well as other tropical diseases.

EXPECTED PRODUCTS (MILESTONES): Effective, non-invasive diagnosis of leishmaniasis.

STATUS/RESULTS TO DATE: Sero-diagnosis of leishmaniasis among PGW veterans Leishmania Serology: Serum analysis was performed on two occasions on our first set of 102 samples with comparable results:

Run #1: Ten of 102 samples yielded a serotest greater than three standard deviations from the mean value obtained from a control group comprising sera of military subjects (unknown exposure history) received from Walter Reed Army Institute of Research (WRAIR). Values obtained from two subjects considered positive controls (one with biopsy-proven L. tropica infection) fell within the range of values exhibited by the 10 seropositive samples.

Run #2: The same 10 of 102 samples yielded a serotest greater than three standard deviations (OD = 0.276) from the mean value obtained from the control group (OD = 0.072) which contained an additional 70 sera obtained from a commercial source in the Boston area. These sera are believed to healthy individuals but their exposure history is unknown. The 10 seropositive samples (OD range of 0.35 to 2.0) fell within the range of values obtained from additional positive control sera obtained from Turkish military subjects with the cutaneous form of L. tropica infection. These individuals were contacted and have been evaluated by Dr. Tom Ward, Infectious Disease Specialist, Portland VAMC. Dr. Ward describes the examinations as being normal and without any indications of active infection by the Leishmania parasite. Whole blood obtained from these same individuals was obtained by Dr. Ward on follow-up and an attempt was made to culture parasites from the WBCs by established means. All such attempts failed. Because there were no signs of active clinical infection, bone marrow aspirates were not collected for parasite culture.

A second set of serum samples were forwarded to Corixa ("Group 2") and similar findings were

observed, i.e., an 8% seropositive rate was observed to a slightly modified Ltr-1 peptide antigen (referred to as "Lt1r-4"). These seropositive individuals have been scheduled for follow-up examinations by Dr. Ward.

A third set of samples (100 new cases/controls, and including 8 serum samples obtained on follow-up examination of our earliest seropositive group) were forward to Corixa for testing in the Spring of this year. In May PEHRC was informed by the company that the firm was no longer interested in development of the leishmania serological-diagnosis test and was in the process of returning the third set of samples to the Portland VAMC.

In summary, approximately 8-10% of subjects (n=202) drawn from a random sample of NW Gulf War veterans showed reproducible evidence of sero-positivity using a synthetic peptide antigen that identifies with the parasite. The antibody titers were extremely low and the test itself remains unproven, primarily due to the lack of adequate controls (i.e., vaccinated military personnel w/o Gulf exposure).

Current Status of Leishmania chemotherapy. The Leishmania are protozoan pathogens which cause infections ranging from cutaneous, muco-cutaneous, to visceral leishmaniasis which afflict millions worldwide primarily in the tropical and subtropical regions of the world. The disease is also of military importance as our Servicemen and women are often employed to endemic areas for training as well as peacekeeping and wartime efforts. Chemotherapy of leishmaniasis relies heavily on the use of pentavalent antimonials that require lengthy courses of treatment at high doses and parenteral administration. It is an antiquated form of heavy metal pharmacology borrowed from the ancient Egyptians. The prototypical antimonial "stibogluconate" suffers from toxic side effects and variable efficacy which is especially poor in treatment of leishmaniasis in patients with AIDS. No orally active or prophylactic antileishmanial agent is available today. Clearly, additional compounds must be identified for treatment of the disease. Ideally, new drugs could be developed with fewer side effects and with broad-spectrum antiprotozoal activity.

Research focus. A rational approach in this search for new drugs is to exploit biochemical differences between the parasite and its mammalian host. One specific example in the case of Leishmania relates to the biosynthesis of heme - a critical prosthetic group for proteins involved in metabolism and electron transport. Like all Trypanosomatids, Leishmania require heme or pre-formed porphyrins as essential growth factors due to a lack of several key enzymes in the heme biosynthesis pathway. In vitro, the heme requirement is met by its addition to the chemically defined medium, while in vivo the parasite must rely on an exogenous supply provided by the host cell. Compounds that selectively block the acquisition of heme or its porphyrin precursors by the parasite should be useful in treatment of the leishmaniasis and other diseases caused by organisms which are reliant on their host cells for procurement of tetrapyrroles.

We have recently identified xanthenes a novel class of antimalarial agents (Ignatushchenko et al., FEBS Letters, 409: 67-73, 1997). Selected xanthenes were shown to form stable, soluble complexes with heme and to block heme polymerization - a key process in the survival of the intraerythrocytic form of the Plasmodium parasite. Considering the specific nutritional requirements of Leishmania, we speculated that these organisms would be exquisitely sensitive to the effects of heme- and porphyrin-complexing xanthenes. We have now documented the antileishmanial activity of xanthenes and provide experimental evidence of their ability to block parasite access to heme. We have evidence that this mode of action may play a role in the antiparasitic action of pentamidine (a drug employed as second line therapy for leishmaniasis and trypanosomiasis and in front-line therapy of PCP) and that our overall drug design scheme may be converging on similar path (manuscript submitted).

Progress since last report:

- Exploitation of parasite heme deficiency ·
- Development of in vitro conditions for invasion of macrophages (i.e., J774 cells) by *L. tropica* isolate 1063. This development makes possible drug testing of our xanthenes vs. the amastigote form. ·
- Investigation by NMR and uv/visible spectroscopy of the binding interactions between heme and 4,5-dihydroxyxanthone (Dissociation constant, K_d , ~ 10⁻⁶M). ·
- Continued synthesis and evaluation of modified xanthenes as antileishmanial agents in vitro vs. promastigotes. In vitro testing vs. amastigotes in macrophages is just underway.
- Computer modeling of disubstituted xanthenes docking onto heme employing the software.

SPARTAN and SCULPT for Silicon Graphics O2 workstation.

- Identification of 4,5-diamidinoxanthone and 4,5-diaminoxanthone for optimized binding to free heme.
- Demonstration that another "diamidine", the clinically useful pentamidine, also complexes heme. The significance of this discovery is described below.
- Biochemical studies are underway to determine the kinetics of ¹⁴C-heme binding to *L. tropica* promastigotes. It is anticipated that the work will lead to mechanistic studies of drug action as well as to purification of the parasite heme transporter. We will employ the intensely colored heme analog, Zn-protoporphyrin, to identify the protein on PAGE gels with the targeted future goal of evaluating the recombinant parasite protein in diagnosis and immunotherapy (vaccine) of Leishmaniasis.

PUBLICATIONS: Ignatushchenko M, Winter R, Hinrichs D, and Riscoe M. Antileishmanial drug design, Chemotherapeutic exploitation of parasite heme dependency. (Under revision). Ignatushchenko M, Winter RW, Riscoe M. Xanthoness as Antimalarial Agents; Stage-specific action and structure-activity relationships. (Submitted).

Kelly JX, Winter RW, Riscoe MK, Peyton DH. Xanthoness as antimalarial agents: Binding interactions between heme analogues and xanthoness. (Submitted).

Ignatushchenko M, Winter RW, Hinrichs DJ, Riscoe MK. Xanthoness as antimalarial agents: Studies of a possible mode of action. FEBS Letters, 409:67-73, July, 1997.

Winter RW, Ignatushchenko M, Ogundahunsi OAT, Cornell KA, Johnson LL, Oduola AMJ, Hinrichs DJ, Riscoe MK. A radical mechanism for potentiation of antimalarial oxidant drugs. Antimicrob Agents Chemother, 41:1449-1454, 1997.

Winter RW, Cornell KA, Johnson LL, Ignatushchenko M, Hinrichs DJ, Riscoe MK. Potentiation of the antimalarial agent rufigallol. Antimicrob Agents Chemother, 40:1408-1411, 1996.

Title: Desert Storm Reunion Survey

Project #: VA-7

Agency: VA

Study Location: VAMC Boston

Project Status: Complete

Research Type: Clinical Research

P.I.: Jessica Wolfe, Ph.D.

Research Focus: Brain & Nervous System, Symptoms/General Health

Start Date (CY): 1991

Est. Completion (CY): 1995

OVERALL PROJECT OBJECTIVE: The Ft. Devens ODS Reunion Survey represents a longitudinal assessment of readjustment of Persian Gulf War veterans returning through Ft. Devens, Massachusetts following their return from the Gulf region.

SPECIFIC AIMS: 1. Describe troop demographics of the Ft. Devens cohort of Persian Gulf War veterans; 2. Examine, both cross-sectionally and longitudinally, family and unit support factors, war-zone exposure, PTSD and psychological distress symptomatology in these veterans; 3. Examine physical health status at Time 2 and explore potential risk factors important in the development of adverse physical health after return from a wartime environment.

METHODOLOGY: Subjects were recruited from a group of approximately 5000 Persian Gulf War veterans who returned home through Ft. Devens, Massachusetts after the Gulf War. The survey at Time 1 constituted a broad range of demographic questions accompanied by an assessment of Post-Traumatic Stress Disorder (PTSD) symptomatology (Mississippi Scale for Combat-related PTSD; Keane, Cadell & Taylor, 1988), general psychological well-being (Brief Symptom Inventory, BSI; Derogatis & Melisaratos, 1983), and combat exposure (Gallop, Laufer & Yager 1981; Rosenheck, 1992), along with queries of other relevant domains including change in work status, current family and social functioning, and post-deployment major life stressors (e.g., death of a loved one, separation or divorce, loss of property, physical or sexual assault) and at Time 2.

additional questions were added to further assess psychological health and physical health symptoms. Individual health symptoms were assessed using the 20-item Health Symptom Checklist (Bartone et al., 1989) which asked subjects to indicate the presence and frequency of symptoms over the past several weeks using Likert-type ratings (0=none, 1=a little, 2=often, 3=very often). In addition, subjects were asked to rate their current physical and psychological health separately ('poor', 'fair', 'good', 'very good' or 'excellent') as well as to rate changes in their physical and psychological health since return from the Persian Gulf ('much worse', 'worse', 'same', 'better' or 'much better').

EXPECTED PRODUCTS (MILESTONES): Data from this investigation is expected to provide an important opportunity to examine patterns of readjustment and the factors that contribute to the changes in the years soon after return from a wartime environment. Results from analyses of Time 1 and Time 2 collected data have been published. Publications in progress include evaluation of reported health symptoms by this cohort of Persian Gulf War veterans at Time 2, examination of reported sexual harassment by the women surveyed, and a longitudinal assessment of PTSD and psychological symptomatology between Time 1 and Time 2.

STATUS/RESULTS TO DATE: Over 84 U.S. Army units (n=2949) were surveyed immediately upon their return to Ft. Devens, Massachusetts (Time 1) between April and July 1991. Persons tested at Time 1 represented approximately 60% of the troops that were deployed to the Gulf from that base, and comparisons between this sample and data available for the Ft. Devens military population at large indicate that survey respondents are generally representative of the base's military population. Examination of nonparticipants at Time 1 indicated random absences for administrative and medical purposes. The original cohort contained 2,709 men and 240 women with a mean age of 30.2 years (SD = 8.6) and 13.2 years of education (SD = 1.8). The majority of troops were Caucasian (87.4%) and reservists (52.2% National Guard; 19.9% Army reservists; 27.9% active duty). Active duty troops had more black members and were significantly younger in age compared to Guard and Reserve Troops. Overall, veterans in the original cohort do not fully reflect military assignments or ethnic and gender compositions of the total U.S. Army or Gulf force (U.S. Government Accounting Office, 1992; communication with Defense Manpower Data Center). Consistent with the Ft. Devens' mission, a higher than average proportion of the original sample had combat support and service support positions during the war, rather than active combat roles.

Approximately 18-24 months after initial testing, veterans were resurveyed (Time 2) to reevaluate their adjustment. This survey repeated all Time 1 measures and queried other relevant domains including work status, family and social functioning, health status, health service use, and intervening life stressors. In the majority of cases, the Time 2 survey was readministered in persons during weekend unit drills. Persons in units that could not be scheduled in a timely fashion were resurveyed using an identical questionnaire sent through the mail. A total of 2,313 persons successfully completed the Time 2 survey (2,119 men, 194 women; Wolfe et al., 1993), yielding an overall response rate of 78%. Analyses of these data show no significant Time 1 differences between Time 2 respondents and non-respondents on most background or outcome measures (e.g., educational level, PTSD symptomatology). However, individuals who did not complete the Time 2 reevaluation were more likely to be younger and on active duty status. Based on veterans' verbal and written comments concerning their health status at Time 2, the Time 3 investigation (now Project #1 (VA-4B) of the Boston Environmental Hazards Center) was designed to reexamine overall adjustment with a special emphasis on physical health and psychological status and to evaluate cognitive functioning by neuropsychological test methods on a subset of the larger cohort. A Time 4 resurvey of the entire Devens cohort is being conducted as part of the study "Female gender and other potential predictors of functional health status among Persian Gulf war veterans (see DoD-52).

PUBLICATIONS: Wolfe J. Preliminary report of reunion survey on Desert Storm returnees. In R. Rosenheck, et al. (eds.), War-zone stress among returning Persian Gulf troops: A preliminary report. Legislative report to Congress. 1991.

Wolfe J, Kelley JM, Buscela ML, Mark WR. Ft. Devens Reunion Survey: Report of Phase I. In R. Rosenheck et al. (eds.). War-zone stress among returning Persian Gulf troops: Final

Report. Legislative report to Congress. 1992.
Wolfe J, Brown PJ, Kelley JM. Reformulating war stress: Exposure and the Persian Gulf War. Journal of Social Issues, 49, 15-31. 1993.

Title: Psychological Test Data of Gulf War Veterans Over Time

Project #: VA-8

Agency: VA

Study Location: VAMC Mountain Home

Project Status: Ongoing

Research Type: Clinical Research

P.I.: Patrick Sloan, Ph.D.

Research Focus: Symptoms/General Health, Brain & Nervous System

Start Date (CY): 1991

Est. Completion (CY): 1999

OVERALL PROJECT OBJECTIVE: (1) To learn more about reactions to combat exposure, stress, and Persian Gulf War-related health concerns, (2) exploration of the relationships among symptoms, demographic variables and veterans' psychological status, (3) to provide intervention, education, and support to Gulf War veterans by providing a) general information about reactions to combat/desert duty, and b) opportunities for individual discussion of experiences and current physical health, (4) administration of standardized psychological questionnaires and instruments, and (5) referral to VA and other community services available for health and other related concerns.

SPECIFIC AIMS: To learn more about psychological stress and physical symptoms experienced by Persian Gulf War veterans.

METHODOLOGY: Initially evaluated 66 Gulf War Marine reservists on a number of psychological instruments within 5 months of Operation Desert Storm using the Mississippi Scale for Desert Storm War Zone Personnel, MMPI-2, Impact of Event Scale, War Stress Inventory-Operation Desert Storm Version, Rorschach test evaluation of DSM-III-R criteria for post-traumatic stress and post-traumatic stress disorder (PTSD). At one year follow-up, the MMPI-2 and Impact of Event Scale were collected on a sub-sample of these Gulf War veterans. At 3-year follow-up, all of the original measures, plus a physical symptom questionnaire selected questions from the Persian Gulf Registry Code Sheet and The Hand Test, were administered to a sub-sample of the original group and a control group of Marine Reservists from the same unit who had never been stationed outside of the U.S. Statistical analyses employed in this study included correlation, paired and independent t-tests, ANCOVA, MANCOVA, discriminant analysis, stepwise discriminant analysis, and repeated measure design ANOVA. Currently, volunteers referred for psychological and neuropsychological evaluation will complete all the measures listed above, the Halstead-Reitan Neuropsychological Test Battery, and questions related to disability status.

EXPECTED OUTCOMES: Assess the utility of psychological instruments in the detection of war-zone stress reactions. Instrument variables associated with response to stressors are expected to be related to DSM-III-R criteria for post-traumatic stress and PTSD.

STATUS/RESULTS TO DATE: The project is currently ongoing, the sample size is increasing and data are being collected. Results to date show differences over time between the initial evaluation data and follow-up, and between initial evaluation and control group on selected variables associated acute distress, and capacity for coping. Findings are being analyzed in relation to the assessment, understanding, and treatment of post-traumatic stress symptomology of PTSD. Continuing to collect data and have several manuscripts submitted for publication.

PUBLICATIONS: Sloan P, Aresenault L, Hilsenroth M. A longitudinal evaluation of the Mississippi Scale for Combat-related PTSD in detecting war-related stress symptomatology. Journal of Clinical Psychology, (54)8:1085-90, 1998.

Sloan P, Aresenault L, Hilsenroth M, Harvill L, Handler L. Rorschach measures of post-traumatic stress in Persian Gulf War veterans: A 3-year follow-up study. Journal of Personality Assessment. 66(1). 54-64. 1996.

Sloan P, Arsenault L, Hilsenroth M, Harvill L. Assessment of non-combat, war-related post-traumatic stress symptomatology: validity of the PK, PS, and IES Scales. *Assessment*, (3)1, 37-41, 1996.

Sloan P, Arsenault L, Hilsenroth M, Harvill L, Handler L. Rorschach measures of post-traumatic stress in Persian Gulf War veterans. *Journal of Personality Assessment*, 64, 397-414. 1995.

Title: Evaluation of Cognitive Functioning in Persian Gulf War Veterans Reporting War-related Health Problems

Project #: VA-9

Agency: VA

Study Location: VAMC New Orleans

Project Status: Complete

Research Type: Clinical Research

P.I.: Patricia Sutker, Ph.D.

Research Focus: Brain & Nervous System, Symptoms/General Health

Start Date (CY): 1994

Est. Completion (CY): 1995

OVERALL PROJECT OBJECTIVE: Addressing a topic of timeliness and importance, this project evaluated cognitive and psychosocial functioning among Operation Desert Storm (ODS) veterans who experienced health problems and physical symptoms following return from the Persian Gulf War zone. The primary objective of the project was to explore environmental, neurotoxic, and psychological correlates of health complaints among these veterans.

SPECIFIC AIMS: The project examined potential differences between veterans reporting high levels of health concern and those reporting low levels of concern on measures of psychological well-being, psychopathology, environmental exposure to toxins and adverse environmental events in the war-zone and prior to war-zone duty, and neuropsychological functioning.

METHODOLOGY: Conducted in cooperation with a research team of psychologists working at the Boston VAMC, study design incorporated a local sample of 78 Persian Gulf returnees who were divided into high and low health concern subsets on the basis of self-reported health status. Participants underwent a clinical diagnostic evaluation that includes administration of an environmental exposure interview; a structured psychodiagnostic interview; a battery of neuropsychological tests selected for their specificity and sensitivity to neurotoxicity and emotional and motivational factors; and self-report inventories assessing mood, emotional well-being, PTSD symptomatology, combat exposure, and physical symptoms. Statistical analysis incorporated a multivariate analysis of variance approach, comparing the two health concern subgroups from both sites on measures of environmental exposure, psychological and neuropsychological functioning, and physical symptoms.

EXPECTED PRODUCTS (MILESTONES): Given current interest in the multiple, yet inadequately diagnosed or explained health problems and symptoms reported by sizable subsets of returnees from the Persian Gulf War, this project will have direct relevance to health care delivery in the VA system nationwide. Study results will be pertinent to development of appropriate clinical diagnostic and treatment services for Persian Gulf returnees and veterans of more recent military operations and objective evaluation of veteran disability and compensation claims in the VA system.

STATUS/RESULTS TO DATE: Data collection at New Orleans site has been completed. All data have been forwarded to the Boston site for multi-site statistical analysis and dissemination of findings.

PUBLICATIONS: none to date

Title: Memory and Attention in PTSD

Project #: VA-10

Agency: VA

Study Location: VAMC New Orleans

Project Status: Ongoing

Research Type: Clinical Research

P.I.: Jennifer Vasterling, Ph.D.

Research Focus: Brain & Nervous System, Symptoms/General Health

Start Date (CY): 1994

Est. Completion (CY): 1999

OVERALL PROJECT OBJECTIVE: Clinical reports have documented that as many as 67-100% of veterans undergoing treatment for war-related post-traumatic stress disorder (PTSD) exhibit deficits in memory and concentration. This project seeks to address the common and potentially debilitating problems of impaired learning, memory, and attention functions reported by veterans suffering PTSD.

SPECIFIC AIMS: Incorporating two separate investigations, this research applies a comprehensive assessment protocol that includes evaluation of psychopathology and neuropsychological functioning in veterans of Vietnam and a younger cohort of Gulf returnees. In particular, the project will provide in-depth description and analysis of learning, memory, and attention functioning among these veterans.

METHODOLOGY: Within each study, comparisons of neuropsychological performances are conducted between community-recruited groups of veterans with PTSD diagnoses related to war-zone duty, veterans with diagnoses of depression, and veterans found to be free of psychopathology. Psychopathology is assessed with structured clinical interview and self-report inventories. Attention, learning, memory, and other cognitive processes are assessed with standardized neuropsychological assessment instruments. Data analysis, conducted separately for the two cohorts, are accomplished using a multivariate of analysis approach.

EXPECTED PRODUCTS (MILESTONES): Because problems with attention, learning, memory, and other cognitive functions can dramatically disrupt social and occupational functioning, as well as hinder treatment efforts of war-related stress and other disorders, studies of neuropsychological sequelae to war trauma are pivotal to understanding the complex behavioral and emotional expression of PTSD, to delivery of appropriate, effective treatment interventions to these veterans, and to exploring long-term psychosocial outcomes in veterans suffering PTSD and served by the VA system.

STATUS/RESULTS TO DATE: To date, this newly funded research has been conducted on a sample of 95 Gulf War returnees. Findings reveal that in comparison to psychopathology-free veterans, veterans diagnosed with PTSD performed significantly more poorly on verbal intellectual tasks including those tasks thought to predict premorbid functioning. Results suggest that intellectual resources, particularly verbal skills, may buffer development of stress-related psychopathology following trauma exposure. Analysis of cognitive data revealed specific PTSD-related deficits in sustained attention, working memory, initial acquisition of new material, and resistance to retroactive interference. In addition, PTSD diagnosis was associated with errors of commission and intrusion. Results are consistent with models of PTSD that emphasize the role of hyperarousal and implicate dysfunction of frontal-subcortical systems. Although data collection with Vietnam veterans is ongoing, this phase of the data collection for the Gulf War cohort is complete. Plans are underway to collect longitudinal neuropsychological data (Time 2) for the Gulf War cohort.

PUBLICATIONS: Vasterling JJ, Brailey K, Constans JI, Sutker PB. Attention and memory dysfunction in posttraumatic stress disorder. *Neuropsychology*, 12:125-133, 1998.

Title: Neuropsychological Functioning in Veterans

Project #: VA-11

Agency: VA

Study Location: VAMC New Orleans

Project Status: Complete

Research Type: Clinical Research

P.I.: Jennifer Vasterling, Ph.D.

Research Focus: Brain & Nervous System, Symptoms/General Health

Start Date (CY): 1992

Est. Completion (CY): 1995

OVERALL PROJECT OBJECTIVE: Complaints of impaired memory and concentration among veterans seeking treatment for post-traumatic stress disorder (PTSD) highlight the importance of systematic evaluation of these cognitive functions among PTSD-diagnosed veterans. The primary objective of this project was to collect pilot data regarding learning, memory, and attention functioning in Persian Gulf returnees reporting PTSD symptomatology.

SPECIFIC AIMS: The specific aim of this project was to compare learning, memory, and attention performances in samples of Persian Gulf returnees with and without diagnoses of PTSD.

METHODOLOGY: Subjects were 15 Persian Gulf War zone veterans diagnosed with military-related PTSD and 27 Persian Gulf War zone veterans found to be free of any PTSD symptomatology. All subjects underwent structured psychiatric interview and neuropsychological assessment using a brief screening battery designed to emphasize attention, learning, and memory functioning. Statistical analyses included a series of univariate analyses of variance (ANOVAs) and univariate analyses of covariance (ANCOVAs) using vocabulary performance as a covariate to compare the two samples on demographic variables and dependent measures of cognitive functioning.

EXPECTED PRODUCTS (MILESTONES): Results of this project impact potentially on comprehensive assessment and treatment services provided to PTSD-diagnosed Persian Gulf War zone veterans.

STATUS/RESULTS TO DATE: Veterans in the PTSD sample were on average older, less educated, and performed more poorly on a vocabulary task used as an estimate of premorbid intellectual sophistication than veterans in the comparison sample. Comparisons of group performances on the neuropsychological tasks revealed that the PTSD sample performed more poorly than the comparison sample on measures of simple and complex attention and word retrieval. When differences in estimated native intellectual skills were controlled for statistically by the inclusion of vocabulary performance as a covariate in a series of ANCOVAs, no significant differences between groups emerged on any of the attention or memory measures. That the effects of PTSD on attention and memory functions could not be parceled out from those attributable to such factors as lower levels of education and premorbid intellectual sophistication does not negate the clinical significance of study findings. Results of this exploratory pilot project complement previous research suggesting that higher levels of education and intellectual functioning may buffer the impact of trauma on the development of subsequent stress-related psychopathology. This phase of the project is complete and has led to the development of a more refined and comprehensive neuropsychological protocol to be administered to a larger sample of Persian Gulf veterans.

PUBLICATIONS: Vasterling JJ, Root L, Brailey K, Uddo M, Sutker PB. Attention and Memory performances in post-traumatic stress disorder. Paper presented at 22nd Annual Meeting of the Internat. Neuropsychological Society, Cincinnati, OH. February 1994.

Title: Psychological Assessment of Operation Desert Storm Returnees

Project #: VA-12

Agency: VA

Study Location: VAMC New Orleans

Project Status: Complete

Research Type: Clinical Research

P.I.: Patricia Sutker, Ph.D.

Research Focus: Brain & Nervous System

Start Date (CY): 1991

Est. Completion (CY): 1997

OVERALL PROJECT OBJECTIVE: This research was designed to determine the psychological

sequelae to war-zone exposure among troops who served in the Gulf during Operation Desert Storm (ODS) compared to troops who were activated during ODS but not deployed to the Gulf.

SPECIFIC AIMS: Objectives of this project were to conduct comprehensive psychological assessments and debriefings among the masses of troops mobilized in support of ODS; identify psychological, somatic, and cognitive symptoms and mental disorders, both early and persistent, that are associated with Gulf war--zone exposure; and explore personal resources and environment factors that may differentiate Gulf--deployed troops exhibiting war-related psychopathology from troops found to be free of psychological disturbances.

METHODOLOGY: Comprehensive psychological assessments were conducted with non-treatment-seeking troops who served in the Gulf and a comparison sample of troops from the same military units who were activated during ODS but not deployed to the Gulf. Follow-up assessments were conducted to assess psychological status over time and ongoing symptomatology. The subject sample includes 1520 military personnel mobilized in support of ODS, including 517 Louisiana National Guard and 1003 Marine, Army, Air Force, and Navy Reserve troops, 194 of whom were members of Quartermaster units assigned graves registration duties. Subjects reported an average age of 29 years at time of initial assessment and completed an average of 13 years of formal education. The percentage of African-American and other minority troops was 43%, and 14% were women. An assessment battery tapping domains of personal resources, stressor characteristics, negative mood states and traits, psychiatric and physical symptoms, and symptoms of posttraumatic stress disorder (PTSD) was administered in regularly-scheduled drill exercises at 6, 8, and 12 months following ODS and at one-year follow-up intervals. An individually-administered comprehensive structured clinical diagnostic interview was conducted with a subset of graves registration troops judged to be at high risk for negative psychological sequelae because of their gruesome Gulf body handling duties. Data analytic strategies for continuous variables included multivariate and univariate analyses of variance, with repeated measures where indicated, and stepwise discriminant function analyses. Chi-square analyses were conducted for categorical variables.

EXPECTED PRODUCTS (MILESTONES): By establishing prevalence estimates for negative sequelae to war--zone duty, identifying troops at high risk for development of war-zone-related psychopathology, and determining factors associated with increased psychological vulnerability to war-zone service, results have implications for development of more effective and efficient strategies and procedures for military training and preparing troops for war-zone exposure, conducting debriefing exercises to facilitate community reentry following war-zone service, and providing mental health treatment interventions specific to war-zone-related psychopathology.

STATUS/RESULTS TO DATE: The project is completed. Comparisons of data collected upon initial assessment among 876 war-zone deployed and 396 non-war-zone deployed troops showed that troops exposed to war-zone duty reported higher levels of depression, anxiety, and somatic preoccupations than did the non-war-zone deployed sample. Specifically, 23% of the war-zone deployed sample showed at least mild levels of clinical depression, and 14% met criteria for PTSD measured by paper-and-pencil instruments. War-zone troops complained more often of somatic discomfort than did non-war-zone troops, citing headaches (22% versus 13%), general aches and pains (20% versus 13%), lack of energy (18% versus 12%), sleep disturbances (17% versus 10%), and common cold or flu (16% versus 11 %). Troops who served in the Persian Gulf described more mental health symptoms as war-zone stress severity increased, and symptom expression among 349 war-zone troops increased over a 1-year follow-up interval on measures of depression, anxiety, anger, physical distress symptoms, and PTSD. Among those who served in the Gulf war-zone, ethnic minority troops were more frequently assigned PTSD diagnoses than were their white counterparts; however, women did not evidence greater vulnerability to negative psychological sequelae to war-zone exposure than men. War-zone-related PTSD diagnoses were associated with low levels of the personality hardiness dimension of commitment, reliance on avoidance as a coping mechanism, low perceived family cohesion, and low satisfaction with available social supports, and scores on these dimensions at initial assessment predicted the presence/absence of PTSD at one-year follow-up with 77% accuracy.

Comprehensive structured clinical diagnostic interviews were administered to a subset of troops

judged to be at high risk for negative psychological outcomes to war-zone trauma by virtue of particularly gruesome war--zone assignments, i.e., graves registration or body handling duties, during the Gulf War. Among 24 Louisiana based troops of the 630th Quartermaster Company who performed Persian Gulf graves registration duties, prevalence of current PTSD was found to be 46%, and rates of comorbid disorders among troops assigned PTSD diagnoses included major depression (55%), alcohol abuse/dependence (27%), depressive disorder NOS (18%), and simple phobia (9%). Findings were replicated and extended by assessments conducted among the Puerto Rico-based 246th Quartermaster Battalion with subsequent comparisons of 40 troops who performed graves registration duties in the Persian Gulf to 20 similarly-trained troops who were not deployed to the Gulf and who had no ODS graves registration exposure. Diagnoses of current PTSD were found in 48% of war-zone deployed troops; whereas, none of the non war zone deployed troops met PTSD criteria. Low rates of pre-ODS deployment psychiatric disorders were found in both subsets of troops. Current comorbid diagnoses among troops who met criteria for PTSD included depressive disorder (37%), alcohol dependence (21%), other anxiety disorder (5%), and polysubstance dependence (5%). One-year follow--up assessments were completed with 17 troops of the 630th Quartermaster Company who were assigned Gulf War graves registration duties, and 47% met criteria for current PTSD. Of the 26 troops of the 246th Quartermaster Battalion who performed war-zone graves registration duties and completed follow-up assessment, 38% evidenced current PTSD. Thus, 42% of troops assessed at follow-up were classified as positive for PTSD two years after war-zone service.

PUBLICATIONS: Brailey K, Vasterling JJ, Sutker PB. Psychological aftermath of participation in the Persian Gulf War. In A. Lundberg (ed). The Environment and mental health: a guide For clinicians. Lawrence Earlbaum: Mahwah, NJ, 83-101, 1998.

Benotsch E, Brailey K, Vasterling JJ, Uddo M, Constans JI, Sutker PB. Longitudinal relationship of individual resource factors to stress-related psychopathology (in press).

Title: Neurobehavioral Aspects of Persian Gulf Experiences: A Pilot Study

Project #: VA-13

Agency: VA

Study Location: VAMC Pittsburgh

Project Status: Complete

Research Type: Clinical Research

P.I.: Gerald Goldstein, Ph.D.

Research Focus: Brain & Nervous System

Start Date (CY): 1994

Est. Completion (CY): 1995

OVERALL PROJECT OBJECTIVE: To collect data on a sample of 50 Persian Gulf Veterans who have returned with physical or psychosocial complaints.

SPECIFIC AIMS: To obtain pilot data to gain objective evidence of impairment of cognitive ability and brain function, or of psychosocial disability.

METHODOLOGY: Subjects were tested in our laboratories with an extensive battery of neuropsychological tests, event-related brain potentials, measures of cardiac and pupillary reactivity, laboratory measures of attention, and various interviews, tests, and questionnaires to evaluate psychosocial status with an emphasis of PTSD. Data were analyzed descriptively and in reference to available literature and normative information. The presence of abnormal findings should provide the basis for further study with a controlled investigation.

EXPECTED PRODUCTS: To obtain neuropsychological, psychophysiological and psychosocial data from 50 Persian Gulf veterans.

STATUS RESULTS TO DATE: A paper describing the neuropsychological test results has appeared in the Journal of the International Neuropsychological Society. The findings are summarized as follows. A neuropsychological investigation of 21 Persian Gulf veterans and 38 demographically matched controls was conducted in order to make a preliminary determination concerning presence of neuropsychological deficits associated with the Persian Gulf War

experience. The neuropsychological test battery consisted of measures of complex attention, memory, and motor skills, previously shown to be sensitive to exposure to environmental toxins. It was found that the Persian Gulf veterans group did not demonstrate substantial impairment, but an impairment index derived from 14 test variables was statistically significantly different from controls in the direction of poorer performance. A second study involving the psychophysiological data is currently being prepared for submission to Biological Psychiatry as a brief report. We found prolonged latencies, without reduction in amplitude, in the P300 component of the auditory event related potential (ERP) in the Persian Gulf veterans. This prolongation was significantly different from demographically matched controls when we only considered the 13 of 19 subjects who had complaints related to cognitive function, such as memory impairment. The remaining 6 subjects had normal latencies. Prior research has shown that increased latencies are associated with decline in cognitive efficiency. We therefore concluded that reported cognitive deficits may not be associated with psychological distress alone. A paper reporting these results is under review by the Journal of the International Neuropsychological Society.

PUBLICATIONS: Goldstein G, Beers SR, Morrow L A, Shemansky WJ, Steinhauer SR. A preliminary neuropsychological study of Persian Gulf veterans. Journal of the International Neuropsychological Society, 2:368-371, 1996.
Steinhauer SR, Beers SR, Goldstein G, Shemansky WJ, Morrow LA. Neurophysiological and neuropsychological characteristics of Gulf War veterans. J Intern Neurop Soc, (abstract) 2:68, 1996.

Title: Vaccine-Mediated Immunity Against Leishmaniasis

Project #: VA-15

Agency: VA

Study Location: VAMC Cleveland

Project Status: Ongoing

Research Type: Mechanistic

P.I.: Frederick Heinzel, M.D.

Research Focus: Leishmaniasis, Prevention

Start Date (CY): 1993

Est. Completion (CY): 1999

OVERALL PROJECT OBJECTIVE: To determine the expression and function of T cell costimulatory molecules during vaccination of mice with Leishmania major antigen and cytokine adjuvants. We hypothesize that distinct costimulatory interactions can be manipulated in vivo to specifically induce or enhance unipolar Th1 CD4+ responses that produce interferon-gamma and thereby protect against disease.

SPECIFIC AIMS: 1. Determine how CTLA4 and CD28 mediate opposing effects on T cell phenotype and disease outcome in disease-susceptible and resistant mice following primary infection or vaccination with Leishmania major. 2. Determine cytokine-dependent and -independent mechanisms by which CTLA4 and CD28 affect disease outcome. 3. Examine the efficacy of activating anti-CD40 antibody as a Th1-selective adjuvant.

METHODOLOGY: We hypothesize that strong CD28 signals are required for Th2 responses in BALB/c mice and that the experimental loss of CTLA4 counter-regulation exacerbates disease. Blocking CD28 should reverse susceptibility and promote protective vaccination. Since anti-CTLA4 Mab treated exacerbates disease in IL-4 deficient mice, we hypothesize that CD28 and CTLA4 mediate these effects by cytokine-independent regulation of CD40 ligand or IL-12 receptor expression during infection and vaccination. We also hypothesize that CD40 activation activates dendritic cells and macrophages to produce factors that promote Th1 selection is response to vaccination. These responses include production of IL-12 and the induction of T cell IL-12 receptor. CD40 activation may also promote dendritic cell expansion and maturation and we will test the alternative hypothesis that increased numbers of dendritic cells will alter the immune phenotype induced by vaccination.

EXPECTED PRODUCTS (MILESTONES): Reagents that block CD28 or that activate CTLA4

could be used to direct Th1/Th2 cytokine responses during antigen-driven cellular immune responses. CD40 stimulating molecules, such as soluble CD40 ligand or anti-CD40 Mab might prove to be useful vaccine adjuvants.

STATUS/RESULTS TO DATE: Blockade of CTLA4 worsens leishmaniasis in BALB/c mice, including mice with deletions in the IL-4 gene. A role of IL-13 or altered IL-12 receptor function is postulated. Treatment with anti-CD40 Mab prevents progressive leishmaniasis in susceptible BALB/c mice and appears to cure established disease as well. Furthermore, anti-CD40 blocks Th2 responses induced by injection with *Schistosoma* egg. Th2 reversal occurs in IL-12 p40 knockout mice, suggesting unique mechanisms for controlling immune deviation towards Th1 type immunity.

PUBLICATIONS: Sang DK, Ouma JH, Whalen CC, King C, Mahmoud AAF, Heinzel FP. Increased levels of soluble interleukin-4 receptor in serum of patients with visceral leishmaniasis. *J Infect Dis* (in press).
Yip HC, Karulin AY, Tary-Lehmann M, Heeger P, Trezza RP, Heinzel FP, Forsthuber T, Lehmann PV. Adjuvant guided type 1 and type 2 immunity. *J Immunol*, 1998 (in press).
Heinzel FP, Rerko RM, Jujer AM, Maier RA. Increased interleukin-2 synthetic capacity parallels disease progression in mice infected with *Leishmania major*. *Infect Immun*, 66:4537-40, 1998.
Heinzel FP, Rerko RM, Jujer AM. Underproduction of interleukin-12 during progressive murine leishmaniasis is due to decreased CD40 activity. *Cell Immunol*, 184:129-142, 1998.
Heinzel FP. From oriental sore to Kala-azar: Clinical and therapeutic aspects of the Leishmaniasis. *Clin Microbiol, Newsletter*, 19:121-6, 1997.
Heinzel FP, Jujer AM, Ahmed F, Rerko RM. The in vivo production and function of IL-12 p40 homodimers. *J Immunol*, 158:4381-8, 1997.
Heinzel FP, Rerko RM, Ahmed F, Jujer AM. Interferon-gamma independent production of interleukin-12 during murine endotoxemia. *J Immunol*, 157:4521-8, 1996.

Title: Protective Immunity in Experimental Visceral Leishmaniasis

Project #: VA-16

Agency: VA

Study Location: VAMC San Antonio

Project Status: Complete

Research Type: Mechanistic

P.I.: Peter Melby, M.D.

Research Focus: Leishmaniasis, Prevention

Start Date (CY): 1994

Est. Completion (CY): 1997

OVERALL PROJECT OBJECTIVE: Characterization of the protective immune mechanisms in experimental visceral leishmania, and identification of the parasite antigens which elicit such protective responses.

SPECIFIC AIMS: Studies of the murine model of visceral leishmaniasis will provide a means to characterize the operative protective immune mechanisms as well as identify parasite antigens which have potential use for human vaccination. Characterization of the protective immune mechanisms in experimental visceral leishmaniasis will be accomplished by studies of the in situ cytokine response to primary infection in susceptible mice, as well as identification of responses associated with the acquisition of immunity. Once the mechanisms associated with protective immunity have been defined the parasite antigens relevant to immunity can be identified. Purified and recombinant *Leishmania donovani* antigens which elicit an in vitro cytokine response which correlates with in vivo immunity will be identified and characterized.

METHODOLOGY: The murine model of visceral leishmaniasis will be used to characterize the mechanisms involved in protective immunity at the tissue level. These studies were performed using a reverse transcriptase polymerase chain reaction methodology for measuring cytokine (IL 4, 11, 6, IL 10, IL 12, IFN- γ) gene expression in skin, lymph node, and spleen. Comparisons

were made between mice infected locally (skin) and systematically (intravenous). Expression of these cytokines in the spleen was also studied at the protein level using immunohistochemical techniques. Because infection of mice with *L. donovani* does not result in a progressive lethal infection, we have also begun to study the immunopathogenesis of VL in the Syrian hamster model which very closely mimics the progressive, fatal disease seen in humans. Because there were no reagents available to study this model, we cloned and sequenced a number of the hamster cytokine genes (IL-2, IL-4, IL-10, IL-12, IFN- γ , TNF α , and TGF- β). These molecular probes were then used to characterize the expression of cytokine mRNAs in active VL in the hamster model. In our work to identify vaccine candidates for this disease, we have cloned recombinant *L. donovani* antigens into a eukaryotic vector for use in DNA immunization studies. Because of the prominent role IL-12 plays in the local containment of *L. donovani* infection through induction of a Th1 response, we have constructed an IL-12 expression plasmid for use as an adjuvant in the DNA immunization studies.

Once the in situ mechanisms are better defined, in vitro correlates of immunity (e.g. a certain cytokine profile) can be identified. Semipurified or purified soluble antigens will then be tested in vitro for their capacity to elicit a proliferative response and protective cytokine profile in splenocytes or Lymph nodes. A recombinant library will also be directly screened using spleen cells from immune animals. This will enable the identification of vaccine candidates which can subsequently be tested in the animal model.

EXPECTED PRODUCTS (MILESTONES): 1. Identification of cytokines associated with control of infection; 2. Identification of cytokines associated with resistance to reinfection; 3. Identification of antigens having the potential to stimulate protective immunity.

STATUS/RESULTS TO DATE: Mice infected with *L. donovani* amastigotes by the intradermal route develop minimal cutaneous swelling, regional lymphadenopathy, but no detectable visceral (hepatic) parasite burden. In contrast, mice infected by the intravenous route develop progressive hepatosplenomegaly and a visceral parasite burden that increases up to 4-6 weeks after infection. To understand the mechanisms associated with the local control of infection and active visceral disease, we have characterized the in situ expression of cytokines (IFN- γ , IL-4, IL-10, and IL-12) in the spleen, draining lymph node, and cutaneous site of inoculation compared to the spleen following systemic inoculation. IL-10 and TGF- β mRNA and protein expression were prominent in the spleens of systemically infected animals. There was significant expression of both IL-10 and IL-12 at the cutaneous site of inoculation starting 7 days after infection. Thus there seems to be a mixed Th1/Th2 type response to primary infection in this model, but local control (skin and draining LN) appears to be associated with markedly increased levels of IFN- γ and IL-12 RNA. These differences may be due to more efficient antigen presentation at the LN level following cutaneous infection (possibly from migration of cutaneous cells), or the induction of immunological tolerance following intravenous infection.

Additional studies were performed using a similar methodology to characterize the immunopathogenesis of VL in the highly susceptible hamster model. In order to study the model, multiple hamster cytokine genes were cloned, sequenced and used as molecular probes to determine the levels of mRNA expression in response to active visceral disease. There was a strong Th1 cytokine expression in the spleen in spite of the progressive nature of disease in this model of lethal infection. IL-10, but not IL-4 expression was markedly increased in response to infection and is likely to play a role in the progressive nature of this disease.

A number of *L. donovani* antigens previously identified as a target of immune T cells have been cloned for study of vaccine candidates. The open reading frames have been cloned into a eukaryotic expression vector, expressed in mouse fibroblasts and macrophages, and the nature of the T cell response directed toward it is being characterized. Mice have been immunized with these constructs and studies to determine their protective efficacy are in progress. The IL-12 expression vector we have constructed will be used as an adjuvant in these immunization studies.

PUBLICATIONS: Melby PC, Yang YZ, Cheng J, Zhao W. Regional differences in the cellular immune response to experimental cutaneous or visceral infection with *Leishmania donovani*. *Infect Immun.* 66:18-27. 1998.

Melby PC, Tryon VV, Chandrasekar B, Freeman GL. Cloning of Syrian hamster (*Mesocricetus auratus*) cytokine cDNAs and analysis of cytokine mRNA expression in experimental visceral leishmaniasis. 1998 (manuscript submitted).

Title: Immunological Evaluation of Persian Gulf Veterans

Project #: VA-17

Agency: VA

Study Location: VAMC Birmingham

Project Status: Complete

Research Type: Clinical Research

P.I.: Michael P. Everson, Ph.D.

Research Focus: Immune Function, Symptoms/General Health

Start Date (CY): 1994

Est. Completion (CY): 1995

OVERALL PROJECT OBJECTIVE: To understand possible contributions of immunological Abnormalities to disease manifestations in Persian Gulf War veterans. The chief complaints of these veterans include arthralgias and central nervous system dysfunctions, i.e., disorders which may have a common immunological basis. The intent of this proposal was to expand upon ongoing medical and neurobehavioral analyses of veterans from the Birmingham VA Medical Center to include an immunologic component in the evaluation of these veterans.

SPECIFIC AIMS: Evaluate potential changes in the immunological status of Persian Gulf War veterans using sensitive biological assays of immune cellular function such as those previously described for use in evaluating chemical sensitivities.

METHODOLOGY: Functional immune integrity of peripheral blood mononuclear cells was tested in subjects (approximately 40) and age- and sex-matched controls (approximately 10). Longitudinal samples were obtained from 2 subjects. Antigenic and mitogenic responses were determined using the recall antigen tetanus toxoid and the T-cell mitogen phytohemagglutinin. Proliferative responses were measured by tritiated thymidine uptake and compared with cultures of unstimulated subject or control cells. Data were reduced and statistically compared using a two-sample, unpaired t test.

STATUS/RESULTS TO DATE: The final number of samples analyzed for immune function in mitogen-induced and antigen-induced proliferative assays included approximately 40 Persian Gulf veterans and 7 age- and sex-matched controls. When these two groups were compared for mitogenic responses, no significant difference was noted ($p > 0.06$). This finding suggested that the subjects and controls had similar T cell function potential since the phytohemagglutinin mitogen is a polyclonal stimulant used to assess potential for T cell responses. However, when these two groups were compared for antigenic response using the tetanus toxoid recall antigen, a significant difference was noted ($p = 0.027$). With less than 50 subjects and less than 10 controls, these data are to be viewed and interpreted only as preliminary findings suggestive of a difference in recall response to antigenic stimulation. More subjects are needed prior to publication or dissemination of these preliminary findings.

PUBLICATIONS: none to date

Title: Chronic Gastrointestinal Illness in Persian Gulf Veterans

Project #: VA-18

Agency: VA

Study Location: VAMC Boston

Project Status: Complete

Research Type: Clinical Research

P.I.: Mark Sostek, M.D.

Research Focus: Symptoms/General Health

Start Date (CY): 1994

Est. Completion (CY): 1996

OVERALL PROJECT OBJECTIVE: Clearly define the most prevalent chronic gastrointestinal symptoms in a unit of Persian Gulf veterans.

SPECIFIC AIMS: 1. Determine and define symptom complex. 2. Look for underlying pathophysiology.

METHODOLOGY: Survey of a single unit of Persian Gulf veterans. Hydrogen breath tests on affected individuals.

EXPECTED PRODUCTS (MILESTONES): Ultimately to identify pathophysiology of the gastrointestinal symptoms.

STATUS/RESULTS TO DATE: Survey completed. Have identified most prevalent symptoms.

Note: Following is quoted from poster accepted for Poster Presentation at Digestive Disease week in San Diego, May 1995, Chronic Gastrointestinal Symptoms In Persian Gulf Veterans; MB Sostek, S Jackson, JK Linevsky, EM Schimmel, BG Fincke; Departments of Medicine and Social Services, Boston Veterans Affairs Medical Center and Boston University School of Medicine, Boston, MA.

Background: Persian Gulf Syndrome is characterized by a constellation of chronic symptoms postdating deployment to the Gulf Region in 1991. Intermittent diarrhea is one of the eight most commonly reported symptoms. The prevalence of non-diarrheal gastrointestinal (GI) symptoms is not well documented. Over the past year, we have received increasing referrals for evaluation of Persian Gulf veterans (PGV) with various GI complaints. The aims of this study were I) to determine the prevalence and spectrum of GI complaints in a representative sample from this population and II) to compare this data to a control group of soldiers not deployed to the gulf region.

Methods: A 4-page questionnaire was mailed to the 92 members of a National Guard Unit deployed to the Persian Gulf Region in 1991 and distributed to 44 members (controls) of the same unit who were not deployed to the Gulf Region in 1991. The questionnaire asked the veterans to grade current severity of 26 GI and 10 non-GI symptoms. The survey also asked veterans to recall occurrence of 5 GI symptoms either during or before the Dessert Storm mission.

Results: 57/92 Persian Gulf veterans (62%) responded to the survey. All 44 of the controls returned the survey. The table below summarized the reported frequency of several GI symptoms in this population:

GI Symptom	Before ODS	After ODS	Controls (Current)
Loose Stool	2/57 (3%)	39/57 (68%)*	4/44(9%)
Abdominal Pain	2/57 (3%)	32/57 (56%)*	3/44 (7%)
Excessive Gas	5/57 (9%)	42/57 (74%)*	10/44 (23%)
Nausea/Vomiting	2/57(3%)	13/57 (23%)	1/44 (2%)
Hematochezia	no data	4/57 (7%)	0/44 (0%)

*p<.0001 compared to symptom frequency before desert storm.

Additional frequently reported GI symptoms among PGV's in this survey include: sensation of incomplete rectal evacuation post defecation 34/57 (60%), and watery bowel movements following episodes of abdominal pain 30/57 (53%). The most frequent non-GI symptoms were: fatigue 46/57

(81%), joint pains 42/57 (74%) and headache 38/57 (67%).

Conclusion: A significant number of veterans from a single National Guard unit, deployed to the Persian Gulf, developed GI symptoms while in that region. The majority of these veterans currently continue to report persistent loose stools, lower abdominal pain, excessive gas and tenesmus. Veterans in the same unit, who were not deployed to the Gulf region, report significantly fewer chronic GI symptoms at the present time. While these symptoms are suggestive of the development of irritable bowel syndrome following Persian Gulf deployment, further studies are needed to better understand the pathophysiology of this combination of chronic GI symptoms.

PUBLICATIONS: Abstract accepted for Poster Presentation at Digestive Disease week in San Diego, May 1995. Sostek M. High prevalence of chronic gastrointestinal symptoms in a national guard unit of Persian Gulf veterans. Am J of Gastroent, Vol 91, (12)2494-2497, 1996.

Title: Psychological Adjustment in Operation Desert Shield/Storm Veterans

Project #: VA-20

Agency: VA

Study Location: VAMC Gainesville

Project Status: Complete

Research Type: Clinical Research

P.I.: Patricia Sohler, Ph.D.

Research Focus: Brain & Nervous System

Start Date (CY): 1991

Est. Completion (CY): 1993

OVERALL PROJECT OBJECTIVE: The study examined the psychological effects of military service in the Persian Gulf during Operation Desert Shield/Storm.

SPECIFIC AIMS: Noncombat reserve and national guard troops who were in the Persian Gulf were compared to activated and nonactivated troops who remained in the United States during the Persian Gulf War. The study determined the difference between the three groups in terms of psychological adjustment and impact on cognitions and feelings about the war.

METHODOLOGY: Subjects were 507 members of National Guard and Reserve units in Northcentral Florida. Data was collected over a two-month time period from July 29 to September 23, 1991. Thus, the data was collected approximately 6 months after the ground war ended, and usually from one to two months after the troops had returned to the United States. Data was collected during the customary monthly weekend drills, and with the endorsement of the units' commanders. For units that had been deployed to the Persian Gulf, troops were also briefed about the potential emotional and family problems associated with post-war adjustment, were provided with information about veterans benefits, and were advised of the availability of readjustment counseling in our VAMC and other facilities in their communities. The order of presentation of these elements of necessity varied from site to site, and sometimes within a site, as it was imperative to oblige the needs of the units' and those of others involved (e.g. Vet Center counselors, veterans benefits counselors).

Subjects were assured of the anonymity of their responses, and were instructed to hand their packets of questionnaires directly to the study's investigators, in an attempt to assuage subjects' concerns that their careers might be jeopardized by their disclosures. However, it is evident that doubts lingered, as some subjects commented on their concerns about how the results might be used, and many failed to provide their names (requested in order to conduct follow-up). Six troops omitted information about their gender as well, and were thus excluded from analysis.

The subjects, tested at different sites, consisted entirely of combat support troops, including engineering units, mobile medical units, transportation units, military police, a quartermaster unit, and a demolition unit. Of the 507 troops included as subjects, 397 were male and 110 were female. Although a minority in the other units, females made up about 50% of the mobile medical units. For the purpose of analysis, subjects are categorized as deployed (i.e. activated and sent to the Persian Gulf during Operation Desert Storm/Shield), activated/not deployed (activated for duty during Operation Desert Storm/Shield but serving in the United States) and not activated

(remaining on non-active status throughout Operation Desert Storm/Shield).

	Males	Females
Deployed	213	75
Not Activated	173	26
Activated/Not Deployed	11	9

Due to the low numbers currently available in the Activated/Not Deployed category, the analysis is restricted to Deployed and Not Activated.

Unit officers provided strong encouragement to their troops to participate in the study, and although it is not possible to know exactly what percentage did actually participate, 90% would be a reasonable estimate. Of those subjects that did return their questionnaire packets, many did not complete the entire protocol, which normally required about one hour to complete. The questionnaires were presented in the following order: 1) demographic questionnaire, 2) combat exposure scale from the War Stress Interview, 3) SCL-90 plus 15 additional items from the Cincinnati Stress Reaction Scales, 4) Gainesville Readjustment Questionnaire, 5) Family Environment Scale, 6) Impact of Events Scale (IES), 7) Work Environment Scale.

RESULTS: Analysis of the data indicated that individuals deployed to the Persian Gulf differed significantly from individuals in reserve and guard units who were not activated during ODS. Specifically, they reported a greater degree of intrusion and avoidance symptoms related to the war, as measured by the IES. ODS veterans who were also veterans of prior wars reported fewer intrusion and avoidance symptoms. Age was not found to be a significant factor. The scores on the Gainesville Questionnaire, postulated to indicate enhanced coping strategies, did not correlate significantly with evidence of superior mental health on the SCL-90-R or the IES. A lack of significant difference between ODS veterans and control group in terms of SCL-90-R scores suggests that the overall psychological health of most of the ODS veterans does not appear to have been affected by the war. However, it is apparent that some subjects had lingering doubts about the possible repercussions of admitting problems, and this is likely to have suppressed some of the SCL-90-R scores, which showed less pathology than non-patient norms in the general population. Additionally, the units available to this investigation were all combat support units, and combat exposure was consequently more limited and less severe than for combat units. Only 156 subjects returned follow-up data, with only 27 completing all 3 follow-ups. An ANOVA conducted to determine if the follow-up group was representative of the larger population resulted in a significant interaction effect, in which ODS veterans who cooperated with the follow-up admitting to higher intrusion and avoidance symptoms on their initial testing. Thus, the follow-up data was concluded to not represent a random sample of the desired population. One subject found the mailed follow-up questionnaires caused discomfort and indicated that she did not want to be contacted further. We abided by her request. No other adverse effects were noted.

PUBLICATIONS: Returning Persian Gulf Troops: First Year Findings, published by Northeast Program Evaluation Center (NEPEC), VAMC, West Haven, Connecticut, 1992.

Title: A Comparison of PTSD Symptomatology among Three Army Medical Units Involved in ODS

Project #: VA-21

Agency: VA

Study Location: VAMC Phoenix

Project Status: Complete

Research Type: Clinical Research

P.I.: Nancy Errebo, Psy.D.

Research Focus: Brain & Nervous System

Start Date (CY): 1992

Est. Completion (CY): 1994

OVERALL PROJECT OBJECTIVE: The objective of the study was to compare three Arizona Reserve Medical Units involved in Operation Desert Storm on PTSD symptomatology one year after the war utilizing the revised Mississippi Scale for Combat-Related PTSD (Keane, Caddell, and Taylor, 1988). One of the groups was deployed to Saudi Arabia(N=42), one to England (N=37), and one to Arizona (N=17).

SPECIFIC AIMS: The hypothesis was that the group deployed to Saudi Arabia would have significantly higher scores than the other two groups.

METHODOLOGY: Subjects consisted of three Arizona Military Reserve Medical Units, one deployed to Saudi Arabia, one deployed to England and one deployed locally. Members of all units included physicians, nurses and other medical specialists.

The group deployed to Arizona (AZ) consisted of 17 subjects, 10 females (58.8%) and 7 males (41.2%). Of the 13 subjects in AZ who provided demographic data, 2 were age 20-29 (15.4%), 5 were 30 to 39 (38.5%), and 6 were 40 to 50 (46.2%). Ages ranged from 20 to 54. The mean age was 38.9. Mean age for females was 34.7. Mean age for males was 43.1.

The group deployed to England (ENG) consisted of 37 subjects, 21 females (56.8%) and 16 males (43.2%). Eleven were age 20-29 (29.7%), 14 were 30 to 39 (37.8%), 9 were 40 to 49 (24.3%), and 1 was 50 to 59 (2.7%). Ages ranged from 21 to 56. The mean age was 33.7. Mean age for females was 34.4. Mean age for males was 33.

The group deployed to Saudi Arabia (SA) consisted of 42 subjects, 20 females (47.6%) and 22 males (52.4%). Twelve subjects were age 20-29 (28.6%), 10 were 30-39 (23.8%), 15 were 40-50 (35.7%) and 5 were 50-59 (11.9%). Ages ranged from 21 to 56. The mean age was 37. Mean age for females was 35.8. Mean age for males was 38.1.

Design, Procedures, and Instruments. These data were collected in the first half of 1992, approximately one year after the end of the Persian Gulf War. At a regular monthly unit meeting, consenting subjects were administered the current revision of the Mississippi Scale for Combat-Related PTSD (Keane, Caddell and Taylor, 1988) and a questionnaire on demographics, substance abuse and effects on family. The validity and reliability of the Mississippi Scale have been established by Keane, Caddell and Taylor (1988). Subjects were invited to write additional comments about the impact of the war and were given an opportunity to volunteer to be interviewed.

The purpose and procedures of the study were described to subjects, and they were informed that they could withdraw from the study at any time prior to publication. They were also informed that participation or refusal to participate will not affect their military careers or subsequent benefits. All responses remained confidential. Data were identified by codes rather than names. Mississippi Scale means of the three groups were analyzed by a one-way Analysis of Variance. The Scheffe Test of Multiple Comparisons was performed post hoc. Subjects' written comments, as well as responses regarding drug and alcohol use, were compiled and summarized but not statistically analyzed. The Semi-Structured Initial Interview for Desert Storm War-Zone Personnel (Litz, Knight, Wolfe, Kaloupek, Quinn, Krinsley, Fisher, Weathers, & Keane, 1991) was administered to ten members of SA by one of the authors. The authors analyzed the data for common themes. Interviewees' descriptions of war-zone stressors are reported in the results section of the paper.

Table 1 Demographics of Interviewees (N=10)

Variable	Males (N=3)	Females (N=7)
Mean age	48.6	39.9
Age range	45-52	22-50

Age range	45-52	22-50
Marital status		
Married	3	4
Single	--	3
Military rank		
Officer	2	5
Enlisted	1	2
Education		
Doctorate	1	--
Masters	--	2
Bachelors	--	2
Some college	2	3
Race		
Caucasian	3	7

RESULTS: Analysis of Mississippi Scale Scores

A one-way Analysis of Variance revealed a significant difference in Mississippi Scale scores among the three groups ($p < .001$). Post hoc analyses revealed that the mean score of the unit deployed to Saudi Arabia (SA) ($M = 83.98$) was significantly higher than that of the unit deployed to England (ENG) ($M = 73.61$) and the unit deployed to Arizona (AZ) ($M = 73.61$), $F(2, 103) = 9.83$, $p < .001$. Also, ENG obtained significantly higher scores than AZ. Means, standard deviations and test results are presented in Tables 3, 4 and 5.

Table 3: Means and Standard Deviations

	Mean	SD	N
SA	83.97	19.58	43
ENG	73.6	15.88	38
AZ	66.00	11.43	25

Table 4: Analysis of Variance Effect

	SS	df	MS	F	P
VAR1	5453.91	2	2726.954	9.83	.001
Within	28570.06	103	277.379	--	--

Table 5: Post hoc Comparison: Scheffe Test: p-levels marginal means for indep. var.

	AZ	ENG	SA
mean=66.00 level code: AZ	X	0.178	0.00
mean=73.61 level code: ENG	0.178	X	0.04
mean=83.98 level code: SA	0.00	0.04	X

Six SA subjects (14%) scored greater than 107, the cutoff score for the diagnosis of PTSD. One of those subjects wrote on the questionnaire that he believed that his distress was caused by his Vietnam experience rather than his ODS experience. One subject in ENG scored over 107 (2.7%). War-Zone Stressors of SA as Described by Interviewees. The unit arrived in Dhahran, Saudi Arabia, on January 4, 1991 and was evacuated on April 4, 1991. Initially billeted in Al Khobar, near the Dhahran Air Base, they were in Al Khobar when the SCUD missile hit. As medical personnel, they were not allowed to carry weapons according to the Geneva Convention, but they were required to pull guard duty both in Dhahran and in the field. They were responsible for setting up their own combat support hospital 10 miles north of the Iraqi border. The closest support hospital to the front lines, they were married with a helicopter unit and received 32% of the war's casualties from both Allied and Iraqi forces. Specific casualties were seen as especially stressful: The first female casualty, abandoned Iraqi children for whom they had no pediatric instruments, and a nurse and physician who were killed when the Claymore mines they had gathered as souvenirs exploded. Nine subjects feared for their lives. Seven said the SCUD missile landing was highly stressful. Two women found the casualties, particularly women and children, extremely stressful. Others said the casualties were not particularly stressful because they saw severe injuries in their daily work.

Noncombat War-Zone Stressors. All subjects said that they were anxious and/or bored waiting for war

to begin. Worries included fear of dying, chemical warfare, the morality of the war, and welfare of family members at home or serving in Saudi Arabia. Six subjects mentioned cultural or environmental stressors. A Jewish man feared torture if taken prisoner. Two women said Saudi men touched them and/or made sexual comments. A woman feared "crazy" Saudi drivers. Two women and two men spoke of heat and the "endless" and "dark" desert. Other stressors were relationships with other unit members, distrust of leaders, poor sanitation, and physical labor.

Deployment and Homecoming Stressors. Deployment stressors included anxiety about leaving family members and disruption of careers. Three women mentioned leaving children as the hardest thing they had ever done. One woman worried about her aging mother. Homecoming stressors included problems and changes in relationships and careers. Three women said that readjustment was more difficult than they had anticipated. A man divorced after the war. A woman had to start over in school. One woman attributed symptoms of diarrhea, fatigue, and irritability to a "parasite". Another had carpal tunnel. All subjects denied exacerbation of previous traumas.

Coping Strategies. Subjects coped with stress by talking to friends, relying on religious faith, reading, calling or writing home, self-talk (e.g., "Calm down." "It will be all right."), playing cards, keeping busy/exercise/physical labor, shopping, joking, eating, writing a journal, remembering home, and romance. Several subjects compared coping strategies of men and women. One woman said that women were supportive of one another and thus had fewer emotional problems than men. Another woman said women talked easily and could cry. She said, "Maybe it seems like you're hysterical, but then you feel better. You get hugged when you cry." She said women negotiated assignments if they didn't get along with tentmates; men accepted assignments they didn't like. She noted that both soldiers who were evacuated were men. One man stated that men "adjusted better" while women complained about "access to phones, the filth, and not getting mail".

Some interviewees compared coping strategies of younger soldiers and older soldiers. Three women in their forties and a 49 year old man stated that younger soldiers had more trouble coping than older ones. Older soldiers "laughed a lot, read a lot, played bridge, [and] didn't dwell on" troubles. The man and two of the women said the younger members engaged in sex to cope with stress. One woman noticed that younger soldiers who coped best kept active. Two women noted that some unit members drank alcohol to cope.

Lasting Psychological Impact of the War. Positive effects of the war included greater faith in self, lasting friends, pride in accomplishments, adventure, and a greater sense of purpose.

Only one subject approached full PTSD. She had a flashback while working in the emergency room and still reacted to loud noises. One man said he had not slept through the night since the war. One woman was emotionally numb. One man was avoidant.

Several interviewees thought that members most affected by the war had quit the Reserves. Several thought that people who had served in Vietnam were more distressed by ODS than other members. A nurse who served in both wars said that better facilities made Vietnam "easier"; "primitive" conditions made ODS very stressful.

Common Themes of Structured Interviews (N=10)

	Number of Subjects	Women	Men
Stressor			
Afraid of dying	10 (100%)	7	3
SCUD landing	7 (70%)	5	2
Seeing severe casualties	2 (20%)	2	0
Waiting for war to begin	10 (100%)	7	3

Waiting for war to begin	10 (100%)	7	3
Cultural/Environmental factors	6 (60%)	3	3
Interpersonal relations	3 (30%)	1	2
Distrust of leaders	3 (30%)	2	1
Hard physical labor	2 (20%)	1	1
Poor sanitation	3 (30%)	3	1
Leaving children	3 (30%)	3	0
Leaving other family members	1 (10%)	1	0
Relationship and/or career disruption	5 (50%)	4	1
War-related physical problems	2 (20%)	2	0
Coping Strategies			
Talking to friends	6 (60%)	4	2
Religion	4 (40%)	3	1
Reading	4 (40%)	2	2
Calling/Writing home	3 (30%)	2	1
Exercise/Physical Labor	3 (30%)	2	1
Self-talk (e.g., "calm down", "I can handle this", "I am strong")	3 (30%)	3	0

"I'm all right")			
Shopping	2 (20%)	1	1
Joking	2 (20%)	1	1
Eating	1 (20%)	1	1
Writing a journal	1 (10%)	1	0
Remembering home	1 (10%)	0	1
Romance	1 (10%)	0	1
Lasting Psychological Impact (At the time of the study)			
Positive effects	6 (60%)	5	1
Confidence/Pride/Purpose	4 (40%)	3	1
Lasting friends	3 (30%)	2	1
Adventure	2 (20%)	1	1
PTSD Symptoms	4 (40%)	2	2
Intrusive thoughts	1 (10%)	1	--
Startle response	1 (10%)	1	--
Sleep disturbance	1 (10%)	--	1
Emotional numbing	1 (10%)	1	--
Flashbacks	1 (10%)	1	--
Avoidance	1 (10%)	--	1

PUBLICATIONS: none to date

Title: Stress Symptoms and Their Causal Attribution in Desert Storm Veterans
Project #: VA-36
Agency: VA

Study Location: VAMC Clarksburg

Project Status: Complete

Research Type: Epidemiology Research

P.I.: Ralph E. van Atta, Ph.D.

Research Focus: Brain & Nervous System

Start Date (CY): 1995

Est. Completion (CY): 1997

OVERALL PROJECT OBJECTIVE: Describe motivational aspects of stress in relationship to war experience.

SPECIFIC AIMS: Determine stress symptoms and their causal attribution in Desert Storm veterans.

METHODOLOGY: Survey methodology: Life stress questionnaire, multiple regression analysis.

EXPECTED PRODUCTS (MILESTONES): Pilot study - may lead to further studies.

STATUS/RESULTS TO DATE: An inquiry was launched into the stress levels of a sample of Desert Storm veterans and the causes to which they attributed their stress levels. The instrument that we used to assess stress levels and the causes to which they were attributed is the life stress questionnaire (LSQ). This device assesses stress level (SL) as the total number of 52 psychiatric symptoms (expressed in layman's language) that are endorsed by the respondent. It assesses attributive causes of this stress level by means of ratings by the respondent of 14 domains of causation. For the purpose of this research, attributive ratings were requested for an additional domain of causation, "after effects of my military experience".

200 veterans on a VAMC Desert Storm registry were randomly selected as respondents. Questionnaires were mailed to them. The follow-up process was complicated since many subjects had moved and left no forwarding address. When this occurred, new subjects were randomly drawn from the registry file. After 8 follow-ups over a 4-month period, 119 questionnaires (59.5 percent return rate) had been accumulated and data analysis was undertaken. These questionnaires were alternately assigned as they had been received to either a trial group (Group T) or a replication group (Group R).

Stress symptoms endorsed by a majority of the subjects in both groups included muscular stiffness and pain, fatigue, restlessness, forgetfulness, and irritability. SL for Group T was 13.25 with an SD of 1.25. For Group R, SL was 15.84, SD 1.43. The leading attributive cause in both groups was After Effects of Military Experience (AEM). For the Trial Group, the mean for this item was 3.41 (on a 5-point scale); for the R Group, the mean for AEM was 3.73.

Multiple regression analyses were run to determine the pattern of attributive causes first in the Group T and then in the Replication Group. This analysis yielded large and highly significant multiple Rs in both groups. Since Rs approach the range that is more typical of reliability coefficients than validity coefficients, it should be mentioned that past experiences with the LSQ suggest that the strength of the coefficients reflects the high consistency of the instrument and, perhaps, in this case, the motivation of the subjects and the meaningfulness of the assignment. In the Group T, $R=.94$, $p<.0001$ with the Social Conflict (SC). In the Group R, $R=.89$, $p<.0001$ with attributive causes including SC, SI, Health and Self, Health and Dependent, and Demands of School or Classwork.

The LSQ has been used in several other studies and it is of interest to remark that the stress levels reported by these Desert Storm veterans are higher than in any of our previous studies. It is also important to consider that although the predominant attribution of the subjects was to AEM, this variable appeared as a predictor only in the Group T. The study was exploratory in nature and therefore the size of our trial and replications groups was smaller than would generally be acceptable when multiple regression methodology is employed. The inductive nature the multiple regression may have resulted in the identification of unique features of either or both Groups. Whether the findings that characterize either group would generalize to a larger registry sample or to Desert Storm veterans as a group is unknown. Had we found more commonality between the predictors for Group T and those for Group R we might have ventured a hypothesis about attributive causation in Desert Storm veterans. But lacking such a commonality, we will point only to the appearance of Social Isolation and Social Conflict in the equations for both groups.

Finally, it should be mentioned that the predominant or most popular attributive cause in both groups was the AEM; the ratings of this variable by the Group R were even higher than for Group T. The failure of the AEM variable to survive in the multiple regression analysis may reflect the greater convergence of opinion of veterans about the impact of their military experience in their present lives. In variance may have resulted in exclusion of these variables in the statistical analysis.

PUBLICATIONS: none to date

Title: Musculoskeletal Symptoms in Gulf War Syndrome

Project #: VA-40

Agency: VA

Study Location: VAMC Long Beach

Project Status: Ongoing

Research Type: Clinical Research

P.I.: Pamela E. Prete, M.D., FACP

Research Focus: Symptoms/General Health

Start Date (CY): 1994

Est. Completion (CY): 1999

OVERALL PROJECT OBJECTIVE: The Gulf War Syndrome is most likely a heterologous group of disorders resulting from the interaction of each veteran's host defense against the variety of environmental, viral, bacterial and stress toxins unique to the Persian Gulf War. One of the more debilitating symptoms of the GWS has been the arthralgia and arthritis. These complaints are relatively common to foreign deployments. A question arises as to whether musculoskeletal complaints are indeed a manifestation unique to Gulf War Syndrome. The project will address a fundamental question about the Gulf War Syndrome: Do the musculoskeletal symptoms in the GWS reflect those of long foreign deployment or are there unique aspects of these symptoms that identify these symptoms as part of Gulf War Syndrome.

SPECIFIC AIMS: 1. Survey deployments for musculoskeletal symptoms via questionnaire; 2. Compare comparative peace-time vs. Gulf War deployments for musculoskeletal symptoms.

METHODOLOGY: Veterans of the Persian Gulf War with and without symptoms of the Gulf War Syndrome and healthy veterans of the same period who did not serve in the Persian Gulf but have been deployed for peaceful missions will receive a questionnaire which, if positive, will be followed by physician interview in the Department of Veterans Affairs Medical Center, Long Beach (VAMCLB) and March Air Force Base, California. Statistical analysis of the comparative features of those examinations will be evaluated for appearance of features that could be related to the other than foreign deployment, i.e., GWS.

EXPECTED PRODUCTS (MILESTONES): 1. Survey and range of musculoskeletal symptoms Secondary to foreign deployments; 2. Comparison of symptoms between foreign deployments and those to GW theater; 3. Statistical analysis based on chi square and regression analysis of independent variables. Review of available data of deployed reservists/veterans with persistent arthritis/arthralgia complaints from GWS.

STATUS/RESULTS TO DATE: 200+ initial questionnaires; 25 comprehensive examinations. Analysis suspended due to insufficient resources.

PUBLICATIONS: none to date

Title: Diarrhea in Persian Gulf Veterans: An Irritable Bowel-Like Disorder

Project #: VA-46

Agency: VA

Study Location: VAMC Gainesville

Project Status: Ongoing

Research Type: Epidemiology Research

P.I.: Charles A. Sninsky, MD

Research Focus: Symptoms/General Health

Start Date (CY): 1995

Est. Completion (CY): 2000

OVERALL PROJECT OBJECTIVE: To compare visceral sensation in normal subjects, patients with irritable bowel syndrome and patients with diarrhea that abruptly began while serving in the Persian Gulf.

SPECIFIC AIMS: a) To determine if Persian Gulf veterans with abdominal pain, diarrhea and bloating with a negative investigative work-up have an underlying visceral hypersensitivity or a generalized decreased pain threshold. b) To examine the heightened visceral sensitivity present in patients with irritable bowel syndrome and compare the data to those obtained for Persian Gulf veterans with diarrhea. c) To investigate whether an acute treatment with octreotide will increase the tolerance to balloon distention of the rectum and extremity immersion in ice water in Persian Gulf veterans, patients, with the irritable bowel syndrome, and controls. d) To determine the effect of octreotide on oral-cecal transit as measured by the lactulose hydrogen breath test.

METHODOLOGY: Study Design: Informed consent is being obtained prior to the start of the trial. A complete medical history and physical exam will be performed. Blood samples will be collected for a pregnancy test when appropriate. Participants will be asked to discontinue all medications for at least 24 hours before the study, to fast for 12 hours, and to take a Fleet's enema 2 hours before the test procedure. Both the ice water tolerance test and the balloon distention tolerance test will be performed in controls, patients with irritable bowel syndrome, and in Persian Gulf veterans both at baseline and after the administration of octreotide 100 mg subcutaneously. Oral-cecal Transit: The effect of octreotide on small bowel transit will be assessed by the hydrogen breath test. Lactulose (10 g) will serve as the carbohydrate substrate for the breath test. Breath hydrogen concentration is measured by gas chromatography every 15 minutes until peak hydrogen excretion occurs. Peak hydrogen excretion is defined as the highest hydrogen concentration before two successive lower concentrations. Peak hydrogen excretion is the point in time at which the lactulose substrate has reached the cecum. Two points on the hydrogen curve will be used to calculate small intestinal transit. The first is the time to an initial sustained increase of hydrogen concentration of ≥ 10 ppm over baseline. The second point is the time to peak hydrogen excretion. Ice Water Tolerance: The ice water tolerance test is performed to determine if patients have a generalized nonspecific lowered pain threshold from a central pathway. All subjects will be asked to insert their right hand into a mixture of ice and water to the wrist point and to hold it there for as long as possible. The time before withdrawing the hand from the water will be measured with a stop watch for each subject. If the hand is not withdrawn before 4 min, the subject will be instructed to withdraw the hand and a value of 240s will be assigned. Balloon Distention Tolerance: The balloon distention test evaluates a specific visceral afferent pathway as a cause of symptoms in patients with diarrhea. After each subject withdraws their hand from the ice water, a 5-min recovery period is observed. A standard anorectal manometry catheter or barostat (modified manometry balloon that measures rectal compliance) will then be inserted into the rectum to a depth of 10-15 cm. In normal subjects, progressive rectal distention initially leads to the perception of pressure, followed by fecal urgency, and then pain. The subjects' response to progressive rectal distention will be quantitated as: 1) first rectal sensation of the balloon, 2) urge to defecate from balloon distention, 3) rectal discomfort from balloon distention and, 4) rectal pain from balloon distention. The rectal balloon will then be distended in a stepwise fashion by adding 20 ml of air every 3 minutes. Unless this represents the subject's maximum tolerable volume, additional 20-ml increments of air will be added at 3-min intervals up to a maximum of 300 ml. The maximum tolerable volume will be defined as the volume at which the subject requests termination of the procedure because of discomfort. If the subject does not report discomfort, a value of 200 ml will be assigned. After baseline measurements of ice water and balloon distention tolerance, all subjects are being brought back another day for octreotide testing. Octreotide 100 mg will be subcutaneously administered. Thirty minutes following the injection, the ice water and balloon distention tolerance will be repeated in exactly the same manner as above and compared to baseline values.

EXPECTED PRODUCTS (MILESTONES): Expected results are that the patients with Persian Gulf diarrhea, as well as irritable bowel syndrome, will have similar rectal sensation, as well as similar rectal urge to defecate, as well as similar discomfort relative to distention. Second, it is not

expected that there should be a difference in ice water tolerance among either of the three groups.

STATUS/RESULTS TO DATE: At this time, 18 patients have been studied (4 normals, 5 irritable bowel syndrome patients, and 9 patients with Persian Gulf diarrhea). Patients who developed chronic diarrhea while in the Persian Gulf have a significantly lower threshold for symptoms of initial rectal sensation and the perception of discomfort with rectal balloon distension. This visceral hypersensitivity in Persian Gulf Veterans is similar to that observed in patients with Irritable Bowel Syndrome. The hydrogen breath test and octreotide protocols have not yet been completed. Additional testing was added to the protocol to include psychological testing. They include the State Anxiety Index, the Beck Depression Index, and the NEO. These non-invasive tests will be done by the subjects on a volunteer basis. The results will be compared among the three groups: Persian Gulf war veterans who had abrupt onset of chronic diarrhea, controls, and subjects with irritable bowel syndrome.

PUBLICATIONS: Gordon JM, Verne GN, Davis RH, Sninsky CA, Gainesville VAMC, Dept of Med Univ of FL, Gainesville, and Dept of Med, MUSC, Charleston SC. Is the diarrhea in Persian Gulf war veterans caused by an underlying visceral hypersensitivity? Biomedicine, May 1996.

Title: Retrospective Verification of Mustard Gas Exposure

Project #: VA-47

Agency: VA

Study Location: VAMC Louisville

Project Status: Ongoing

Research Type: Clinical Research

P.I.: Jerald L. Hoffman, Ph.D.

Research Focus: Chemical Weapons

Start Date (CY): 1997

Est. Completion (CY): 2000 Diagnosis

OVERALL PROJECT OBJECTIVE: Determine if there is a correlation between mustard gas exposure and human reproductive and developmental problems.

SPECIFIC AIMS: 1. To establish a GC/MS method for analysis of the mustard gas adduct with the N-terminal valine of hemoglobin. 2. To analyze blood samples from Persian Gulf War veterans collected within eight months of potential exposure to chemical weapons. 3. To analyze blood samples from military personnel at the Bluegrass Army Depot collected during routine health surveillance. 4. To survey Persian Gulf War veterans and military personnel working with chemical agents for reproductive difficulties and for developmental problems in their children. 5. To search for a correlation of verified exposure to mustard gas with reproductive and developmental problems. 6. To examine cross-linked hemoglobin as a more sensitive marker of mustard gas exposure. 7. To further characterize the endogenous protection against mustard gas afforded by S-methylation by the human enzyme, thioether methyltransferase.

METHODOLOGY: 1) to establish a GC/MS method for analysis of the mustard gas adduct with the N-terminal valine of hemoglobin; 2) to analyze blood samples from Persian Gulf War veterans collected within eight months of potential exposure to chemical weapons; 3) to analyze blood samples from military personnel at the Bluegrass Army Depot collected during routine health surveillance; 4) to survey Persian Gulf War veterans and military personnel working with chemical agents for reproductive difficulties and for developmental problems in their children; and 5) to search for a correlation of verified exposure to mustard gas with reproductive and developmental problems.

EXPECTED PRODUCTS (MILESTONES): This research will be helpful in defining the role of mustard gas exposure in the Persian Gulf War 'syndrome' of which many VA patients complain.

STATUS/RESULTS TO DATE: Progress to date has involved the chemical synthesis of valine hemoglobin mustard adducts derivatized for gas chromatography/mass spectroscopic analysis

(GC-MS). These include deuterium-labeled forms which will be used as internal standards for GC-MS analysis of hemoglobin samples. We are presently beginning to standardize the analysis of these reagents in the GC-MS.

A second approach to determining exposure to mustard gas has been initiated. Since mustard gas is bifunctional it has the potential to cross-link the various pairs of subunits of hemoglobin. Such cross-linked dimers could be detected by the appearance of a 32 kDa band (2x16 kDa subunits) in western blots of hemoglobin probed with anti-Hb antibody. This method potentially has much higher sensitivity than adduct analysis since chemiluminescent detection can be used. We have also expanded the project to include studies of the endogenous protection against mustard gas provided by the enzyme, thioether methyltransferase. This enzyme methylates the S atom in thioethers including mustard gas analogs which renders them nonreactive as alkylating agents. We originally discovered this enzyme in mice, and cloned and sequenced the cDNA from that organism. Using the mouse cDNA as a probe the human thioether methyltransferase cDNA has been cloned from a liver library. Base sequence analysis revealed only one amino acid difference from the mouse. This indicates that our rabbit antiserum against the mouse enzyme can also be used for studies of the tissue distribution of human thioether methyltransferase by western blotting, and such experiments are in progress. Northern blotting of human tissue mRNA using mouse thioether methyltransferase cDNA as a probe has shown expression of the mRNA only in human liver.

PUBLICATIONS: none to date

Title: Cross-Sensitization as a CNS Model for Gulf War Chemical Intolerance

Project #: VA-48

Agency: VA

Study Location: VAMC Tucson

Project Status: Ongoing

Research Type: Clinical Research

P.I.: Iris R. Bell, MD, PhD.

Research Focus: Environmental Toxicology

Start Date (CY): 1998

Est. Completion (CY): 2001 Brain & Nervous System Interactions

OVERALL PROJECT OBJECTIVE: To test the neural sensitization/cross-sensitization hypothesis for acquired chemical intolerance (ACI) in Persian Gulf veterans and appropriate controls and to determine individual differences in vulnerability to this process. Neural sensitization is the progressive amplification of a given response over the course of repeated, intermittent exposures to a particular stimulus. As many different classes of stimuli (e.g. chemicals, stress, drugs, microbial toxins) can initiate and elicit sensitized responding, cross-sensitization between multiple pre-War, War, and post-War factors could account for the clinical pictures in certain PGW veterans.

SPECIFIC AIMS: 1. Evaluate patterns of responsibility over time of four groups of veterans to repeated jet fuel or sham laboratory exposures, using blood pressure, heart rate, eyeblink (spontaneous and acoustic-startle induced) reactions as outcome measures. The four groups will include three groups of PGW veterans i) ill with increased chemical intolerance attributed to military service; ii) ill without increased chemical intolerance; iii) healthy, without increased chemical intolerance; and an additional group of healthy Gulf War era veterans. 2. Assess for cross-sensitization between jet fuel or sham exposures and non-War related chemical exposures (i.e., perfume). 3. Compare veterans by groups and by degree of laboratory sensitization for premorbid individual difference characteristics (host and environment) that would enhance vulnerability to sensitization.

METHODOLOGY: The primary between-groups, six-session study of veterans (Ntotal = 200) will compare ill PGW veterans who report increases in acquired chemical intolerance since the Gulf War with three age-, sex-, and education-matched control groups (ill PGW veterans without increased ACI, healthy PGW veterans, healthy era veterans) for current baseline sensitization and laboratory-induced sensitizability and cross-sensitization of neurobehavioral responses (e.g.,

spontaneous eyeblink, acoustic startle, reaction times, vigilance task performance, blood pressure, heart rate) to chemical and nonchemical (e.g., noise) stimuli.

The second study will compare premorbid individual difference variables drawn from previous animal and human research that may contribute to susceptibility to neural sensitization in the four groups and in subjects who did versus those who did not show consistent objective evidence of physiological sensitization and cross-sensitization. Data analyses will utilize (1) repeated measure, multivariate analyses of variance and covariance and (2) discriminant analysis and logistic regression involving the individual difference measures. Women and Hispanics will be oversampled because of evidence of being overrepresented, respectively, on the national Persian Gulf War Registry and among those on the Tucson VAMC PGW Registry reporting wartime environmental contaminant exposures.

EXPECTED PRODUCTS (MILESTONES): Completion of 50 participants between 4/98 to 3/99; 90 participants between 4/99 to 3/00; 60 participants and final data analysis, report, and Publications in 4/00 to 3/01. Data from this sensitization/cross-sensitization proposal will provide an empirical approach to testing for objective abnormalities in PGW veterans not seen in studies designed from a classical toxicology perspective using similar substances. This work could point to a multifactorial pathway by which sensitization-related disorders initiated by chemicals, stress, drugs, microbial toxins, or a combination of these factors during the Gulf War might contribute to the longitudinal development of nonspecific somatic complaints as well as of certain medical conditions common in veterans, notably hypertension and its vascular sequelae. Findings will provide a rationale for systematic pharmacological interventions.

STATUS/RESULTS TO DATE: Ongoing. Subject recruitment has recently begun. No results are yet available.

PUBLICATIONS: Bell IR, Warg-Damiani L, Baldwin CM, Walsh M, Schwartz GE. Self-reported chemical sensitivity and wartime chemical exposures in Gulf War veterans with and without decreased global health ratings. *Military Medicine*, 163:725-32, 1998.

Title: Sensitivity to Pyridostigmine Bromide (PB): Persistent Neural Dysfunction

Project #: VA-49

Agency: VA

Study Location: VAMC East Orange

Project Status: Ongoing **Research Type:** Mechanistic

P.I.: Richard J. Servatius, Ph.D.

Research Focus: Pyridostigmine Bromide, Brain & Nervous System

Start Date (CY): 1998

Est. Completion (CY): 2002

OVERALL PROJECT OBJECTIVE: Determine long term of effects of PB on physiology and behavior.

SPECIFIC AIMS: STUDY A will determine the pharmacokinetics of PB in SD and WKY rats. We will determine if there are strain differences in the rate of clearance from portal and jugular vein administration of C14-labeled PB. We will also determine if there are strain differences in the degree and duration of inhibition of BuChE activity. STUDY B will compare the effects of two cholinesterase inhibitors, edrophonium (EDRO) and neostigmine (NEO) to PB on measures of erythrocyte (E-) cholinesterase activity, BuChE activity, and startle responding. Unlike NEO and PB, EDRO does not affect BuChE. We will determine: a) whether the effects of PB are mediated through BuChE activity, and b) whether the appearance of delayed-onset startle sensitization is specifically related to PB. STUDY C will provide evidence that persistent startle sensitization after exposure to prophylactic levels of PB in rats with abnormal BuChE activity is mediated by central, as opposed to peripheral, nervous system dysfunction. To determine whether central cholinergic activity mediates the delayed-onset startle sensitization observed after prophylactic PB treatment in WKY rats, we will perform two similar experiments. In Experiment 1, we will address muscarinic receptor mediated cholinergic activity. WKY rats will be given PB, PB and atropine (central and peripheral activity). PB and methylnatropine (peripheral activity). the antagonists alone. or tan

water. In Experiment 2, we will address nicotinic neurotransmission. WKY rats will be given PB, PB and mecamylamine (MEC, centrally active), PB and hexamethonium (HEX, peripherally active), the antagonists alone, or tap water. For both experiments, startle responses will be measured 1, 8, and 15 days after the end of treatment. Blood samples for BuChE determination will be obtained the day before PB treatment, 4 days after the onset of PB treatment, and the day after the end of PB treatment. In Experiment 3, SD and WKY rats will be given PB treatment or water for 7 consecutive days. Rats will be sacrificed either on the last day of treatment or 8 days after the end of treatment. We will determine AChE activity in the cervical spinal cord, hippocampus, cortex, and brain stem, as well as blood BuChE activity. STUDY D will determine how long enhanced startle responsivity lasts after appearance and the nature of the enhanced responsivity in rats with abnormal BuChE activity. In Experiment 1, WKY and SD rats will be treated with either PB or tap water for 7 consecutive days. The acoustic startle response will be measured beginning 1 day after the end of treatment and continuing every week until normal startle amplitudes are detected in two consecutive weeks. Separate groups of SD and WKY rats will be tested only on the 15th day after the end of treatment to determine whether enhanced responsivity is related to repeated testing. In Experiment 2 WKY and SD rats will be treated as in Experiment 1 except that the single-intensity protocol will be used for behavioral testing. In both experiments blood samples for BuChE determination will be obtained the day before PB treatment, 4 days after the onset of PB treatment, and the day after the end of PB treatment. STUDY E will attempt to produce delayed-onset startle sensitization in rats with otherwise normal BuChE activity. In Experiment 1, half of each strain, WKY and SD rats, will be given a single session of tailshock stress. Following stress, rats will be given either 7 consecutive days of PB or tap water and vehicle. Acoustic startle responses will be measured 1 day after the end of PB treatment and every week thereafter for 4 weeks. STUDY F will directly evaluate the scavenger hypothesis as a possible mechanism for the persistent startle sensitization in WKY rats. In Experiment 1, WKY rats will be given 7 consecutive days of either PB, PB with FSB AChE, FSB AChE or tap water. Startle responses will be measured 1, 8, and 15 days after the end of treatment.

METHODOLOGY: see above.

EXPECTED PRODUCTS (MILESTONES): Studies A and B will be completed in Year 1, Studies C in Year 2, Studies D and E in Year 3, and Study F in Year 4.

STATUS/RESULTS TO DATE: We have determined that treatment with PB results in an persistently exaggerated startle response in Wistar-Kyoto rats. This rat strain also has inherently low butyryl cholinesterase (BuChE) activity, a scavenger of PB. These effects of PB on the appearance of exaggerated startle responses is dose-dependent (Servatius, et al., JPET; in press). Exposure to inescapable stress also persistently lowers BuChE activity, however, the effects of stress and PB treatment are not additive (Servatius et al., submitted). Most recently, we have demonstrated that stressed Sprague-Dawley (common laboratory strain) rats treated with PB have lower brain AChE activity, suggesting that exposure to stress allows PB to penetrate the brain.

PUBLICATIONS: none to date

Title: Neuropsychological findings in a sample of Operation Desert Storm veterans

Project #: VA-50

Agency: VA

Study Location: VAMC Detroit

Project Status: Complete

Research Type: Clinical Research

P.I.: Bradley Axelrod, Ph.D.

Research Focus: Brain & Nervous System

Start Date (CY): Est. Completion (CY): 0

OVERALL PROJECT OBJECTIVE: Objectively evaluate the subjective reports of changes in the cognitive functioning of returning Gulf War veterans.

METHODOLOGY: Volunteers for a single Army reserve unit were given a comprehensive

neuropsychological and psychological evaluation using clinical tools according to each measures' standardized instructions. In response to ongoing complaints of memory, attention, and problem-solving difficulties among veterans of Operation Desert Storm and Shield (ODSS), a sample of 44 male veterans of ODSS underwent a comprehensive neuropsychological evaluation.

EXPECTED PRODUCTS (MILESTONES):

STATUS/RESULTS TO DATE: Deficits relative to normative data were observed only on finger dexterity (Grooved Pegboard, bilaterally) and the Stroop Color and Word Test. Those with impaired Pegboard performance had lower performance on other tasks requiring psychomotor speed. Those with impaired Stroop had significantly lower motor and set-shifting performance. Scores of both impaired groups were higher on many clinical and supplemental scales of the MMPI. Despite subjective cognitive complaints reported in 39% of the overall sample, veterans with cognitive complaints differed from their peers primarily in greater psychological distress as depicted on the MMPI. The data represented as preliminary clinical findings. This evaluation was exploratory in nature and two major weaknesses included the small sample size and that the veterans were volunteers. The data should not be misinterpreted as generalizable to all ODSS veterans. The most difficult aspect of interpreting the neuropsychological and psychological data relates to lack of other external data, including possible causes of the symptoms. The prospective study with objective neuropsychological data of individuals who have had a known exposure to specific toxins is an important next step in clinical research.

PUBLICATIONS: Axelrod BA, Milner IB. Neuropsychological findings in a sample of Operation Desert Storm veterans. J of Neuropsychiatry and Clinical Neurosciences, 9:23-28, 1997.

Title: Psychobiological Assessment of Desert Storm Veterans

Project #: VA-51

Agency: VA

Study Location: VAMC Cincinnati

Project Status: Ongoing

Research Type: Clinical Research

P.I.: Dewleen Baker, M.D.

Research Focus: Brain & Nervous System, Symptoms/General Health

Start Date (CY): 1992

Est. Completion (CY): 2002

OVERALL PROJECT OBJECTIVE: While prior studies show that combat veterans with posttraumatic stress disorder (PTSD) report more physical symptoms than veterans without PTSD, the link between PTSD and somatic complaints in Persian Gulf War veterans (PGWVs) is yet to be evaluated.

SPECIFIC AIMS: We examine the relationship of PTSD and somatic complaints in PGWVs, specifically to determine if PGWVs exhibit PTSD effects similar to those observed in veterans of other conflicts and to delineate the specific somatic complaints accompanying the PTSD symptoms in the Gulf War group.

METHODOLOGY: A questionnaire booklet was completed by 188 PGWVs, of whom half were patients in a veterans health screening clinic and half were non-treatment-seeking volunteers on active duty. The booklet included the Combat Exposure Scale, the Mississippi Post-Traumatic Stress Disorder Scale (MPTSD), and a subjective symptom-based health questionnaire.

EXPECTED PRODUCTS (MILESTONES):

STATUS/RESULTS TO DATE: The 24 PGWVs (12.8%) with PTSD (MPTSD score >116) reported more combat exposure ($P=.02$) and a greater number of physical symptoms ($P=.001$) than other PGWVs. Fatigue, nausea, muscle aches, dizziness, back pain, stomach ache, and numbness were much more likely to be reported by those with PTSD (MPTSD score >116) than by those without PTSD (MPTSD score <95). Physicians examining PGWVs should be alert to the possibility of PTSD in this group and that those with PTSD are more likely to report physical symptoms that may overlap with those in Persian Gulf syndrome. Consequently, mental health screening is essential since for those veterans with PTSD diagnosis of other coexisting

conditions may be confounded and early effective treatment of their PTSD may be delayed. Also, given the increased reporting of certain symptoms by those with PTSD, those seeking the cause of Persian Gulf syndrome should control for PTSD when determining the symptom cluster that may constitute this condition.

PUBLICATIONS: Baker DG, Mendenhall CL, Simbartl LA, Magan LK, Steinberg JL. Relationship between posttraumatic stress disorder and self-reported physical symptoms in Persian Gulf war veterans. Arch Intern Med, 157:2076-78, 1997. Kellner M, Baker DG, Yehuda R. Salivary Cortisol in Operation Desert Storm Returnees. Bio Psych, 849-850, 1997.

Title: Spouses and Children Program

Project #: VA-53

Agency: VA

Study Location: VAMC Denver

Project Status: Ongoing Under Public Law 103-446, Section 107 the VA was authorized to provide medical examinations to any individual; who: a. Is the spouse or child of a veteran, is listed in the Persian Gulf War Veterans Registry established under Public Law 102-585, Section 702; and is suffering from illness or disorder. b. Is suffering from, or may have suffered from, an illness or disorder (including a birth defect, miscarriage, or stillbirth) which cannot be disassociated from the veteran's service in the Southwest Asia theater of operations. c. Has granted VA permission to include in the Registry relevant medical data from the evaluation. Under this program, a eligible Gulf War veterans would receive examinations at the local VA Medical Center, and the examinations of spouses and children would be conducted under contract at the affiliated university medical center. This program will continue until September 30, 1998 or a maximum of \$2.0 m has been expended.

PUBLICATIONS: none to date

Title: Follow-up of Psychological and Neurocognitive Gulf War Outcome: Relation to Stress

Project #: VA-54

Agency: VA

Study Location: VAMC New Orleans

Project Status: New

Research Type: Clinical Research

P.I.: Jennifer Vasterling, Ph.D.

Research Focus: Brain & Nervous System, Symptoms/General Health

Start Date (CY):

Est. Completion (CY): 2003

OVERALL PROJECT OBJECTIVE: Continuation of project #VA-11 "Memory and Attention in Posttraumatic Stress Disorder." The purposes of this continuation are to examine psychopathology and cognitive functioning longitudinally in Gulf War veterans and to extend examination of neuropsychological functioning in Gulf War veterans with and without PTSD.

SPECIFIC AIMS: 1) To conduct longitudinal investigation of psychological outcome (psychopathology, negative mood states, and health complaints) in a large sample of deployed and nondeployed Gulf War veterans previously examined at this site; (2) to conduct longitudinal investigation of PTSD-related neurocognitive deficits in a sample of Gulf War veterans previously examined by our group; and (3) to expand neuropsychological investigation of PTSD through cross-sectional experimental methods targeting hierarchical (global-local) visual processing after threat versus neutral stimuli.

METHODOLOGY: To achieve these aims, this project incorporates follow-up evaluation of psychopathology, negative mood states, and health complaints, as well as examination of the extent to which stressor characteristics, early symptom expression, personal resources, and intervening stress are associated with long-term outcome, in a sample of over 800 deployed and 200 nondeployed Gulf War veterans previously evaluated at this site. In addition. cognitive

functioning will be examined longitudinally by conducting follow-up neuropsychological evaluation among 70 PGW veterans, categorized into three diagnostic groups (PTSD, no mental disorder, subthreshold PTSD/anxiety, mood disorder), who previously underwent such assessment at the NOVAMC. The specific neuropsychological battery will emphasize attention and memory functions. Global-local visual processing in PTSD will be examined in 60 Gulf War veterans (20 with PTSD, 20 without mental disorder, 20 with subthreshold PTSD or anxiety/mood disorders) on a computer administered reaction time task after undergoing each of two conditions (nonthreat, neutral imagery and threat, stress imagery) counterbalanced for order.

EXPECTED PRODUCTS (MILESTONES): Follow-up evaluation of psychopathology is expected to expand knowledge regarding the longitudinal outcome of stress-related disorders among war-zone veterans. In particular, the project will provide information regarding possible early predictive variables for subsequent outcome including stressor characteristics, early symptom expression, intervening life events, and personality, coping style and other individual difference variables. Longitudinal investigation of PTSD-related neurocognitive impairment is expected to provide information relevant to theories of PTSD that implicate neurobiologic alterations associated with the development and maintenance of psychopathology following stress exposure. More specifically, knowledge may be gained regarding the extent to which disorder chronicity alters neurocognitive deficit patterns and the extent to which cognitive deficits are linked to PTSD symptom expression. Cross-sectional study of global-local visual processing biases in PTSD under aroused and non-aroused conditions will address cognitive processing style within PTSD as well as hemispheric specialization of emotion and arousal more generally.

STATUS/RESULTS TO DATE: Project not yet initiated.

PUBLICATIONS: none to date

Title: Antibiotic Treatment of Gulf War Veterans' Illnesses

Project #: VA-55

Agency: VA

Study Location: VAMC PerryPoint/multiple sites

Project Status: Ongoing

Research Type: Clinical Research

P.I.: Sam Donta, M.D.

Research Focus: Treatment, General Health

Start Date (CY):

Est. Completion (CY): 2002

OVERALL PROJECT OBJECTIVE: This study's primary objective is to determine whether a 12 month course of doxycycline treatment in deployed Gulf War veterans with GWI improves patients' functional status compared to placebo. Major secondary objectives are 1) to determine whether doxycycline treatment reduces symptoms of GWI, 2) to determine whether doxycycline treatment converts Mycoplasma (+) patients to mycoplasma (-) status, and 3) to determine if the benefits of 12 months doxycycline treatment endure after termination of treatment.

SPECIFIC AIMS: see objectives.

METHODOLOGY: This study is a 30 month, prospective, randomized, double-blind clinical trial. Patients will be randomized to one of two treatment groups: 1) patients treated with doxycycline for 12 months and, 2) patients given placebo for 12 months. Each patient will receive study medication for 12 months and will be followed for an additional six months after the termination of medications. Patients will be seen monthly during the medication phase and at 18 months. Major evaluations will be completed at baseline and at 3, 6, 9, 12, and 18 months. 450 GWI patients from 30 medical centers will be entered into the study over a one-year recruitment period. With 18 months of follow-up on each patient, total study duration is 30 months. All veterans deployed to the Gulf War between August 1990 and August 1991 will be eligible. Patients will be considered to have GWI if they have at least two of three symptoms (fatigue, musculoskeletal pain, neurocognitive dysfunction) that began after August 1990 and that have lasted more than six months and up to the present. GWI patients meeting any of the exclusion criteria will not be entered. Patients assigned doxycycline will receive 300 mg/day. All patients will be provided with

a potent sunblock preparation for protection from common drug-related photosensitivity.

EXPECTED PRODUCTS (MILESTONES): The primary outcome measure is improvement in the Physical Component Scale (PCS) of the SF-36V at follow-up relative to baseline. The primary endpoints will be the proportion of patients with more than a seven unit increase in the PCS at 12 months.

STATUS/RESULTS TO DATE: Currently planning selection of sites.

PUBLICATIONS: none to date

Title: A Randomized, Multi-Center, Controlled Trial of Multi-Modal Therapy in Veterans with Gulf War Illnesses

Project #: VA/DoD-1D*

Agency: DoD

Study Location: VAMC West Haven/multiple sites

Project Status: Ongoing

Research Type: Clinical Research

P.I.: Sam Donta, M.D.

Research Focus: Treatment, Symptoms/General Health

Start Date (CY): 1998

Est. Completion (CY): 2002

*Same as Project VA/DoD-1V This Project is currently being planned as a part of the VA Cooperative Studies Program.

OVERALL PROJECT OBJECTIVE: This clinical trial will study Gulf War veterans who have unexplained chronic medical symptoms such as pain, fatigue, and/or cognitive difficulties. Patients will be randomized to one of four groups: 1) Cognitive Behavioral Therapy (CBT) plus aerobic exercise, 2) aerobic exercise alone, 3) CBT alone, and 4) usual and customary care. The primary outcome will be a clinically meaningful improvement in the Physical Component Summary (PCS) scale of the SF-36V at one year relative to baseline.

SPECIFIC AIMS: see objectives.

METHODOLOGY: A cohort of 1356 patients (339 per group) who satisfy an operational definition of Gulf War Illnesses will be enrolled from 20 VA medical centers over a 18-month period. The target sample size can be achieved in one year with 20 sites enrolling 68 patients each. All patients will be followed for one year and outcomes will be measured at 3 months (immediately following the end of treatment), 6 months and 12 months. Given the rate of referrals of patients with these symptoms to the VA medical centers, we envision no difficulty in recruiting this many study patients.

EXPECTED PRODUCTS (MILESTONES): The primary hypothesis of this study is that multi-modal therapy will significantly improve clinical outcomes in veterans with Gulf War Illnesses. The primary endpoint is the proportion of patients improved at one year relative to baseline on the PCS scale of the SF-36V. Patients with more than a 7-point increase is used, instead of changes in the actual PCS scale, because it represents a significant improvement and, hence, is more clinically meaningful than just a change in value.

STATUS/RESULTS TO DATE: Currently planning the selection of sites.

PUBLICATIONS: none to date

Title: A Randomized, Multi-Center, Controlled Trial of Multi-Modal Therapy in Veterans with Gulf War Illnesses

Project #: VA/DoD-1V*

Agency: VA

Study Location:

Project Status: Ongoing

Research Type: Clinical Research

P.I.: Sam Donta, M.D.

Research Focus: Treatment. Symptoms/General Health

Start Date (CY): 1998

Est. Completion (CY): 2002

*Same as Project VA/DoD-1D This Project is currently being planned as a part of the VA Cooperative Studies Program.

OVERALL PROJECT OBJECTIVE: This clinical trial will study Gulf War veterans who have unexplained chronic medical symptoms such as pain, fatigue, and/or cognitive difficulties. Patients will be randomized to one of four groups: 1) Cognitive Behavioral Therapy (CBT) plus aerobic exercise, 2) aerobic exercise alone, 3) CBT alone, and 4) usual and customary care. The primary outcome will be a clinically meaningful improvement in the Physical Component Summary (PCS) scale of the SF-36V at one year relative to baseline.

SPECIFIC AIMS: see objectives.

METHODOLOGY: A cohort of 1356 patients (339 per group) who satisfy an operational definition of Gulf War Illnesses will be enrolled from 20 VA medical centers over a 18-month period. The target sample size can be achieved in one year with 20 sites enrolling 68 patients each. All patients will be followed for one year and outcomes will be measured at 3 months (immediately following the end of treatment), 6 months and 12 months. Given the rate of referrals of patients with these symptoms to the VA medical centers, we envision no difficulty in recruiting this many study patients.

EXPECTED PRODUCTS (MILESTONES): The primary hypothesis of this study is that multi-modal therapy will significantly improve clinical outcomes in veterans with Gulf War Illnesses. The primary endpoint is the proportion of patients improved at one year relative to baseline on the PCS scale of the SF-36V. Patients with more than a 7-point increase is used, instead of changes in the actual PCS scale, because it represents a significant improvement and, hence, is more clinically meaningful than just a change in value.

STATUS/RESULTS TO DATE: Currently planning selection of sites.

PUBLICATIONS: none to date

Title: Follow-Up investigation of troops exposed to nerve agents at Aberdeen Proving Ground (Pilot)

Project #: VA/DoD-2DA*

Agency: DoD

Study Location: Aberdeen Proving Ground

Project Status: Complete

Research Type: Epidemiology Research

P.I.: Research Focus: Chemical Weapons

Start Date (CY): 1996

Est. Completion (CY): 1999

*Same as Project VA/DoD-2VA

OVERALL PROJECT OBJECTIVE: Between 1955 and 1975, the U.S. Army enrolled 6,720 soldiers in an experimental exposure program of chemical warfare and other agents at the Edgewood Arsenal, Maryland. In 1980 the Army asked the National Research Council (NRC) to study the possible long term health effects of these exposures. A three-volume report was issued, the last volume dealing with the current health status of test subjects, including 1,581 men exposed to anticholinesterase compounds such as GA (tabun), GB (sarin), GD (soman), GF, and VX. The report indicated that "the limited information available from the follow-up on these soldiers does not permit definitive conclusions regarding the nature and extent of possible long-term problems resulting from chemical exposure at Edgewood." A pilot study was undertaken to determine whether follow-up of these test subjects is feasible and whether it would provide useful information.

Taking the suggestion of the NRC committee, we propose to survey by telephone the Edgewood subjects who were exposed to anticholinesterase agents for the more common OP exposure-associated outcomes; neurological deficits, particularly peripheral nerve disease, and neuropsychological impairment, including sleep disorders, anxiety, and depression.

SPECIFIC AIMS:

METHODOLOGY: The Edgewood subjects who were unexposed to chemical agents will serve as the first control group. However, this is not an ideal control group because the original Edgewood protocol did not include random assignment of subjects to treatment and control groups. In particular, it is thought that healthier men were more likely to have been assigned to chemical exposure groups and less healthy men to the control group. To counteract the effect of this putative assignment bias, a second control group consisting of men who were exposed to chemical agents other than anticholinesterases will also be included in the follow-up. We are proposing a screen by telephone questionnaire of all exposed subjects and controls for neurological deficit and neuropsychological impairment, including sleep disorders, anxiety, and depression.

EXPECTED PRODUCTS (MILESTONES):

STATUS/RESULTS TO DATE: MFUA has discussed the results of this pilot study with the Neurology Dept and the Washington DC VA Medical Center and the Minneapolis MN VA Medical Center. They have agreed to work together if a full-scale study is funded. MFUA will also consult with the staff of the Board on Environmental Studies and Toxicology, National Research Council, with regard to toxicological matters. New project is listed as DoD-93.

PUBLICATIONS: none to date

Title: Follow-Up investigation of troops exposed to nerve agents at Aberdeen Proving Ground (Pilot)

Project #: VA/DoD-2VA*

Agency: VA **Study Location:**

Project Status: Complete

Research Type: Epidemiology Research

P.I.: Research Focus: Chemical Weapons

Start Date (CY): 1996

Est. Completion (CY): 1999

*Same as Project VA/DoD-2DA

OVERALL PROJECT OBJECTIVE: Between 1955 and 1975, the U.S. Army enrolled 6,720 soldiers in an experimental exposure program of chemical warfare and other agents at the Edgewood Arsenal, Maryland. In 1980 the Army asked the National Research Council (NRC) to study the possible long term health effects of these exposures. A three-volume report was issued, the last volume dealing with the current health status of test subjects, including 1,581 men exposed to anticholinesterase compounds such as GA (tabun), GB (sarin), GD (soman), GF, and VX. The report indicated that "the limited information available from the follow-up on these soldiers does not permit definitive conclusions regarding the nature and extent of possible long-term problems resulting from chemical exposure at Edgewood." A pilot study was undertaken to determine whether follow-up of these test subjects is feasible and whether it would provide useful information.

Taking the suggestion of the NRC committee, we propose to survey by telephone the Edgewood subjects who were exposed to anticholinesterase agents for the more common OP exposure-associated outcomes; neurological deficits, particularly peripheral nerve disease, and neuropsychological impairment, including sleep disorders, anxiety, and depression.

SPECIFIC AIMS:

METHODOLOGY: The Edgewood subjects who were unexposed to chemical agents will serve as the first control group. However, this is not an ideal control group because the original Edgewood protocol did not include random assignment of subjects to treatment and control groups. In particular, it is thought that healthier men were more likely to have been assigned to chemical exposure groups and less healthy men to the control group. To counteract the effect of this putative assignment bias, a second control group consisting of men who were exposed to chemical agents other than anticholinesterases will also be included in the follow-up. We are proposing a screen by telephone questionnaire of all exposed subjects and controls for neurological deficit and neuropsychological impairment, including sleep disorders, anxiety, and depression.

EXPECTED PRODUCTS (MILESTONES):

STATUS/RESULTS TO DATE: MFUA has discussed the results of this pilot study with the Neurology Dept and the Washington DC VA Medical Center and the Minneapolis MN VA Medical Center. They have agreed to work together if a full-scale study is funded. MFUA will also consult with the staff of the Board on Environmental Studies and Toxicology, National Research Council, with regard to toxicological matters. New project is listed as DoD-93.

PUBLICATIONS: none to date

Title: Patterns of Pre-Persian Gulf War Illness and Health Care Seeking, Pilot Study

Project #: VA/DoD-2VB*

Agency: VA

Study Location:

Project Status: Complete

Research Type: Epidemiology Research

P.I.:

Research Focus: Symptoms/General Health

Start Date (CY): 1996

Est. Completion (CY): 1998

*Same as Project VA/DoD-2DB

OVERALL PROJECT OBJECTIVE: To answer the question whether there are patterns of illness and the patterns of use of the Department of Defense (DoD) outpatient care during the year prior to deployment to the Persian Gulf War (PGW) in personnel who subsequently developed poorly defined illnesses different from the patterns of controls?

SPECIFIC AIMS:

METHODOLOGY: Conducting extensive searches of relevant literature and researching various methods of obtaining medical records; Data abstraction and categorization.

EXPECTED PRODUCTS (MILESTONES):

STATUS/RESULTS TO DATE: Approvals have been obtained from the NRC Governing Board and from the NRC Institutional Review Board. Permission to access records has been received from: VA BIRLS, the Army Personnel Center, and the Army Surgeon General's Office (SGO). We have developed a short study protocol, produced case definitions, and created an algorithm for identifying cases from the PGHR and the CCEP as well as for controls. A database containing a record of all cases and controls has been created in Paradox and SAS. An algorithm for acquiring medical records has also been developed. A second request for claims folders has also been mailed. A registry match of records to determine those that are located at the National Personnel Records Center (NPRC) has been completed.

We have obtained over 1,100 medical records and have begun the abstracting process. It is predicted that the pilot will be completed and the data analyzed by the end of the year. No plan for a full study can be constructed until the completeness of record ascertainment by PGHR or CCEP/control status is known.

PUBLICATIONS: none to date

Title: Patterns of Pre-Persian Gulf War Illness and Health Care Seeking, Pilot Study

Project #: VA/DoD-2DB*

Agency: DoD

Study Location:

Project Status: Complete

Research Type: Epidemiology Research

P.I.:

Research Focus: Symptoms/General Health

Start Date (CY): 1996

Est. Completion (CY): 1998

*Same as Project VA/DoD-2VB

OVERALL PROJECT OBJECTIVE: To answer the question whether there are patterns of illness and the patterns of use of the Department of Defense (DoD) outpatient care during the year prior to deployment to the Persian Gulf War (PGW) in personnel who subsequently developed poorly defined illnesses different from the patterns of controls?

METHODOLOGY: Conducting extensive searches of relevant literature and researching various methods of obtaining medical records; Data abstraction and categorization.

STATUS/RESULTS TO DATE: Approvals have been obtained from the NRC Governing Board and from the NRC Institutional Review Board. Permission to access records has been received from: VA BIRLS, the Army Personnel Center, and the Army Surgeon General's Office (SGO). We have developed a short study protocol, produced case definitions, and created an algorithm for identifying cases from the PGHR and the CCEP as well as for controls. A database containing a record of all cases and controls has been created in Paradox and SAS. An algorithm for acquiring medical records has also been developed. A second request for claims folders has also been mailed. A registry match of records to determine those that are located at the National Personnel Records Center (NPRC) has been completed.

We have obtained over 1,100 medical records and have begun the abstracting process. It is predicted that the pilot will be completed and the data analyzed by the end of the year. No plan for a full study can be constructed until the completeness of record ascertainment by PGHR or CCEP/control status is known.

PUBLICATIONS: none to date

Title: VA/DoD Core funding of the Medical Follow-up Agency

Project #: VA/DoD-2D*

Agency: DoD

Study Location:

Project Status: Ongoing

P.I.:

Start Date (CY): 1993

Est. Completion (CY): 0

*Same as Project VA/DoD-2V

This Program is a joint program funded by VA and DoD to provide the Medical Follow-up Agency with core funding to maintain its operational capabilities to perform epidemiological research on veterans. The MFUA maintains a number of important veterans databases. VA and DoD consider this Program a vital adjunct to its Gulf War programs. It provides VA and DoD with a vehicle by which outside expert epidemiological skills can be quickly brought to bear to conduct small scale pilot projects to test the feasibility of new ideas, and to provide recommendations and proposals to VA and DoD for the conduct of more extensive projects. This Program was authorized under P.L. 102-585, and initially served to fund the IOM/MFUA study panel "The Health Consequences of Military Service during the Persian Gulf War", which produced its final report in September 1996.

PUBLICATIONS: none to date

Title: VA/DoD Core funding of the Medical Follow-up Agency

Project #: VA/DoD-2V*

Agency: VA

Study Location:

Project Status: Ongoing

P.I.:

Start Date (CY): 1993

Est. Completion (CY): 0

*Same as Project VA/DoD-2D

This Program is a joint program funded by VA and DoD to provide the Medical Follow-up Agency with core funding to maintain its operational capabilities to perform epidemiological research on

veterans. The MFUA maintains a number of important veterans databases. VA and DoD consider this Program a vital adjunct to its Gulf War programs. It provides VA and DoD with a vehicle by which outside expert epidemiological skills can be quickly brought to bear to conduct small scale pilot projects to test the feasibility of new ideas, and to provide recommendations and proposals to VA and DoD for the conduct of more extensive projects. This Program was authorized under P.L. 102-585, and initially served to fund the IOM/MFUA study panel "The Health Consequences of Military Service during the Persian Gulf War", which produced its final report in September 1996.

PUBLICATIONS: none to date

Appendix A: project funding

TABLE OF SPENDING FOR GULF WAR VETERANS' ILLNESSES RESEARCH

Research Working Group

Persian Gulf Veterans Coordinating Board

March 1, 1999

NOTE: These notes and the attached table replace and supersede Appendix A-2 of the *Annual Report to Congress: Federally Sponsored Research on Gulf War Veterans' Illnesses for 1997* issued March, 1998.

General

All entries for research funding reflect money centrally committed to researchers (both intramural and extramural) to carry out the specific projects. These funds do not cover operational costs for administration, infrastructure, etc. Each department allocates these costs in slightly different ways making it difficult to accurately account for these funds. For example, in VA the research appropriation does not pay for clinician/investigator salaries. By law those funds must come from the patient care appropriation.

A "blank" funding entry generally reflects years in which a project was not active (e.g. it had not started or it had come to an end).

Some multiyear projects receive all of their funding in the fiscal year of the authorization and appropriation. For those, the dollars authorized and appropriated are shown for that fiscal year. The remaining funding entries show \$0 for the years that the project is active. Some intramural projects/programs are supported out of operational costs. For those projects \$0 is entered for the funds in the fiscal years that the project is active.

Programs consisting of multiple projects are represented in one of two ways depending on how funds are centrally allocated:

Funds centrally allocated to the program: These programs are shown in the table as a main program indicated by a project designation such as DoD-1, and projects in the program as DoD-1A, DoD-1B, etc. All funds are shown under the main program. Blank funding entries are shown for the individual projects.

Funds centrally allocated to projects within a program: These programs are only indicated by their projects without a main program identifier. For example, VA-2A and VA-2B.

Entries indicated by a "00" (e.g. DoD-00) are initiatives or activities for which funds have

been set aside. Generally, these may involve projects that are under peer review; projects approved but under negotiation; or set asides allocated for a new initiatives. In FY'99, \$19 M has been set aside for the new DoD Program Element Initiative for Gulf War veteran's illnesses research.

Funds listed under FY'99 are only projections at this time.

Specific

DoD-4 is part of a larger US Army study (DoD-23) conducted at Walter Reed Army Institute of Research. All funding is combined into project DoD-23. DoD-8A and 8B are also parts of a larger US Army study and all funding is combined and shown under program DoD-8.

Funds for DoD-8A, 8B, and 12 have been combined under DoD-12.

In the Report to Congress for 1997, funds for DoD-7B were broken out by level of effort in each fiscal year instead of representing the fiscal year in which funds were committed to this effort. This has been corrected in the current table.

In the Report to Congress for 1997 the table did not show that an additional \$127,000 was expended on DoD-16 in FY'97. This has been corrected in the present report.

The table in the Report to Congress for 1997 indicated a projected \$2 million to be spent on DoD-18 in FY'98. The actual figure was \$290,000. This has been corrected in the current table.

The funding for DoD-66 has been updated in the present table to accurately reflect the fiscal year appropriations from which funds were expended.

HHS-3 was funded from the FY'91 appropriation, which is not included in this accounting.

HHS-4 was funded from the FY'93 appropriation, which is not included in this accounting.

Funds for VA-1 for FY'94 through FY'97 represent an aggregate of funds for both the VA Mortality Study and the VA National Survey of Persian Gulf Veterans. Beginning in FY'98 VA-1 reflects continuation of the VA Mortality Study. Beginning in FY'98 VA-2A, 2B, and 2C reflect funding for separate components of the VA National Survey of Persian Gulf Veterans.

A category has been created for projects and programs jointly funded by VA and DoD.

The portion of a project funded by DoD ends in a "D" and the portion of a project funded by VA ends in a "V".

[Department of Defense Funding](#)

[Department of Health and Human Services Funding](#)

[Department of Veterans Affairs Funding](#)

[VA/DoD Programs Funding](#)

Research on Gulf War Veterans' Illnesses

Department of Defense Funding

<i>Project #</i>	<i>Title</i>	<i>Status</i>	<i>FY'94</i>	<i>FY'95</i>	<i>FY'96</i>	<i>FY'97</i>	<i>FY'98</i>	<i>94-98 Total</i>	<i>Projected FY'99</i>
DoD-00	1999 DoD Program Element Initiative	Pending						\$0	\$19,000,000

DoD-1	Epidemiologic Studies of Morbidity Among Gulf War Veterans: A Search for Etiologic Agents and Risk Factors	Ongoing	\$725,000	\$1,010,000	\$2,250,000	\$2,000,000	\$2,654,000	\$8,639,000	\$1,500,000
DoD-1A	A Study of Symptoms Among 1500 Seabees	Complete						\$0	
DoD-1B	A Comparative Study of Hospitalizations among Active Duty Personnel who Participated in the Gulf War and Similar Personnel who did not	Complete						\$0	
DoD-1C	A comparative study of pregnancy outcomes among gulf war veterans and other active-duty personnel	Complete						\$0	
DoD-1D	Adverse Reproductive Outcomes in Gulf War veterans	Ongoing						\$0	
DoD-1E	Seabee Health Study	Ongoing						\$0	
DoD-1F	A Comparison of Nonfederal Hospitalization Experience Among Veterans in California who have separated from active service	Complete						\$0	
DoD-1G	Prevalence of Congenital Anomalies Among Children of Persian Gulf War Veterans.	Ongoing						\$0	
DoD-2	Physiological and Neurobehavioral Effects in Rodents from Exposure to Pyridostigmine, Fuels, and DEET (short title: Toxicity of Simulated PGW Exposure)	Complete	\$90,000	\$0	\$0	\$0		\$90,000	
DoD-4	The General Well-Being of Gulf War Era Service Personnel from the States of Pennsylvania and Hawaii: A Survey	Complete	\$0					\$0	
DoD-7A	Health Risk Assessment of Embedded Depleted Uranium: Behavior, Physiology, Histology, and Biokinetic Modeling	Complete	\$897,000	\$0	\$0	\$0	\$0	\$897,000	
DoD-7B	Carcinogenicity of Depleted Uranium Fragments	Ongoing	\$897,814	\$0	\$0	\$0	\$0	\$897,814	\$0

DoD-8	Research on Diagnostic Tools for Leishmaniasis	Ongoing	\$773,000	\$895,000	\$652,000	\$695,000	\$694,000	\$3,709,000	\$0
DoD-8A	Serologic Diagnosis of Viscerotropic Leishmaniasis (VTL)	Complete						\$0	
DoD-8B	Development of a Leishmania Skin Test Antigen (LSTA)	Ongoing						\$0	
DoD-9	Identification of the Genetic Factors Which Control Tropism in Leishmania	Complete	\$0	\$150,000	\$0	\$0	\$0	\$150,000	
DoD-10	Pyridostigmine Synergistic Toxicity	Complete	\$42,000	\$44,000				\$86,000	
DoD-11	Male/Female Differential Tolerances to Pyridostigmine Bromide	Complete	\$549,000	\$359,000	\$0	\$0		\$908,000	
DoD-12	Forward Deployable Diagnostics for Infectious Diseases	Ongoing	\$520,000	\$1,162,000	\$439,000	\$932,000	\$803,000	\$3,856,000	\$0
DoD-13	Effects of Persian Gulf War Service on Military Working Dogs	Ongoing	\$0	\$0	\$97,000	\$200,000	\$120,000	\$417,000	\$200,000
DoD-14	Risk Factors Among US Army Soldiers for Enrolling on the Department of Veterans Affairs Gulf War	Complete	\$120,000	\$0	\$0	\$0		\$120,000	
DoD-15	Comparative Mortality Among US Military Personnel Worldwide During Operations Desert Shield and Desert Storm	Complete	\$20,000	\$0				\$20,000	
DoD-16	Kuwait Oil Fire Health Risk Assessment	Complete	\$345,000	\$137,000	\$50,000	\$127,000		\$659,000	
DoD-17	Retrospective Studies Involving Military Use of Pyridostigmine as a Pretreatment for Nerve Agent Poisoning	Complete	\$21,000					\$21,000	
DoD-18	Kuwait Oil Fires Troop Exposure Assessment Model (TEAM)	Ongoing	\$68,000	\$151,000	\$770,000	\$193,000	\$290,000	\$1,472,000	\$295,000
DoD-19	Persian Gulf Veterans Health Tracking System	Ongoing		\$25,000	\$0	\$0	\$450,000	\$475,000	\$450,000
DoD-20	A Statistical Study Correlating Illnesses in Gulf War Veterans to Battlefield Locations of Afflicted US Army	Complete	\$50,000	\$0				\$50,000	

	Iraq-Kuwait War; Part 1: Method to Relate Troop Deployment and Illnesses in GWVs								
DoD-21	Study of Variability In Pyridostigmine Inhibition of Blood Cholinesterases in Healthy Adults and Individuals With Symptoms Following Participation in Operation Desert Storm	Complete	\$38,000	\$100,000	\$0	\$0		\$138,000	
DoD-22	Chronic Organophosphorus Exposure and Cognition	Complete	\$375,000	\$0	\$0	\$0	\$0	\$375,000	
DoD-23	Acute and Long-Term Impact of Deployment to Southwest Asia on the Physical and Mental Health of Soldiers and their Families	Complete	\$621,000	\$688,000	\$764,000	\$985,000		\$3,058,000	
DoD-30	Epidemiological Studies Persian Gulf War Illnesses, PG Women's Health Linkage Study	Ongoing		\$779,000	\$0	\$0	\$0	\$779,000	\$0
DoD-31	Dysregulation of the Stress Response in the Persian Gulf Syndrome	Ongoing		\$971,000	\$0	\$0	\$0	\$971,000	\$0
DoD-32	Neuropsychological Functioning in Persian Gulf Era Veterans	Ongoing		\$353,000	\$0	\$0	\$0	\$353,000	\$0
DoD-33	Effects of Pyridostigmine in Flinders Line Rats Differing in Cholinergic	Ongoing		\$354,000	\$0	\$0	\$0	\$354,000	\$0
DoD-34	Characterization of Emissions from Heaters Burning Leaded Diesel Fuel in Unvented Tents	Ongoing		\$283,000	\$0	\$0	\$0	\$283,000	\$0
DoD-35	Feasibility of Investigating Whether There is a Relationship Between Birth Defects and Service in the Gulf War	Ongoing		\$427,000	\$10,000	\$63,000	\$0	\$500,000	\$0
DoD-36	Fatigue in Persian Gulf Syndrome-Physiologic Mechanisms	Ongoing		\$416,000	\$138,000	\$0	\$0	\$554,000	\$0
DoD-37	Neurobehavioral and Immunological Toxicity of Pyridostigmine, Permethrin, and DEET in Male and Female Rats	Ongoing		\$934,000	\$5,000	\$0	\$0	\$939,000	\$0

DoD-38	Diagnostic Antigens of Leishmania tropica	Complete		\$612,000	\$0	\$0	\$0	\$612,000	
DoD-39	A Controlled Epidemiological and Clinical Study into the Effect of Gulf War Service on Servicemen and Women of the United Kingdom Armed	Ongoing		\$865,000	\$0	\$28,400	\$155,000	\$1,048,400	\$0
DoD-40	Psychological and Neurobiological Consequences of the Gulf War Experience	Ongoing		\$264,000	\$0	\$0	\$0	\$264,000	\$0
DoD-41	Evaluation of Muscle Function in Persian Gulf veterans	Ongoing		\$906,000	\$0	\$0	\$0	\$906,000	\$0
DoD-42	The Symptomatic Persian Gulf Veterans Protocol: An Analysis of Risk Factors with an Immunologic and Neuropsychiatric Assessment	Ongoing			\$700,000	\$0	\$0	\$700,000	\$0
DoD-44	Investigation of Seminal Plasma Hypersensitivity Reactions	Ongoing			\$634,000	\$0	\$0	\$634,000	\$0
DoD-45	Physical and Emotional Health of Gulf War Veterans Women	Ongoing			\$100,000	\$100,000	\$0	\$200,000	\$0
DoD-46	Exploratory Data Analysis with the CCEP	Complete			\$60,000	\$100,000		\$160,000	
DoD-47	Study of Mycoplasmal Infections in Gulf War Veterans	Complete			\$112,000	\$0	\$0	\$112,000	
DoD-48	Assessment of Genomic Instability via Chromosome 7 Inversion Frequency in a Symptomatic Cohort of Gulf War Veterans vs Selected Control Groups	Complete			\$74,000	\$0		\$74,000	
DoD-49	Diagnosis and Dosemetry of Exposure to Sulfur Mustard: Development of Standard Operating Procedures and Exploratory Research on Protein Adducts	Ongoing			\$927,000	\$0	\$0	\$927,000	\$0
DoD-50	Toxicokinetics of O-Ethyl S-(2-Diisopropylaminoethyl) Methylphosphonothioate [(+)-VX] in Rats, Hairless Guinea Pigs and ..	Ongoing			\$699,000	\$0	\$0	\$699,000	\$0

	Identification of Metabolic Pathways								
DoD-51	Transgenic Engineering of Cholinesterases: Tools for Exploring Cholinergic Responses	Ongoing			\$864,000	\$0	\$0	\$864,000	\$0
DoD-52	Female Gender and Other Potential Predictors of Functional Health Status Among Persian Gulf War veterans	Ongoing	\$414,000	\$0	\$0	\$0	\$0	\$414,000	\$0
DoD-53	Long-Term Effects of Subclinical Exposures to Sarin	Ongoing			\$1,000,000	\$400,000	\$0	\$1,400,000	\$0
DoD-54	Assessment of Subchronic Neurobehavioral and Neuropathologic Effects in Rats	Ongoing			\$315,000	\$100,000	\$0	\$415,000	\$0
DoD-55	Low-Level Exposure to GB Vapor in Air: Diagnosis/Dosimetry, Lowest Observable Effect Levels, Performance-Incapacitation, and Possible	Ongoing			\$500,000	\$136,000	\$0	\$636,000	\$0
DoD-56	Low-Level Sarin Neurotoxicity and Its Modulation by Pyridostigmine	Ongoing			\$685,000	\$100,000	\$0	\$785,000	\$0
DoD-57	Physiologic Effects of Stress in Gulf War Veterans	Ongoing				\$909,000	\$0	\$909,000	\$0
DoD-58	Illness Among Persian Gulf War Veterans: Case Validation Studies	Ongoing				\$2,208,000	\$0	\$2,208,000	\$0
DoD-59	Pyridostigmine-induced Neurodegeneration: Role of neuronal Apoptosis	Ongoing				\$625,000	\$0	\$625,000	\$0
DoD-60	Butyrylcholinesterase Genetic Variants in Persons with Gulf War Illness	Ongoing				\$125,000	\$0	\$125,000	\$0
DoD-61	Neurophysiologic and Neuropathologic Effects in Monkeys of Low Level Exposures to Sarin, Pyridostigmine, Pesticides, and Botulinum Toxoid	Ongoing				\$1,586,000	\$0	\$1,586,000	\$0
DoD-62	Sarin and	Ongoing				\$201,000	\$0	\$201,000	\$0

	Interaction under Physical Stress: Neurotoxic Effects in								
DoD-63	PGW Veterans: Epidemiological and Clinical Evidence for Residual Organophosphate Neurotoxicity	Ongoing				\$1,626,000	\$0	\$1,626,000	\$0
DoD-64	Individual Differences in Neurobehavioral Effects of Pyridostigmine	Ongoing				\$1,900,000	\$18,516	\$1,918,516	\$0
DoD-65	Multi-Disciplinary Pathophysiologic Studies of Neurotoxic Gulf War-Related Neurologic Syndromes	Pending				\$3,000,000	\$0	\$3,000,000	\$0
DoD-66	Testing for Mycoplasma Infection: Criterion Validity and Replicability of Nucleoprotein Gene Tracking and Forensic Polymerase Chain Reaction Tests	Ongoing			\$49,940	\$100,000	\$63,825	\$213,765	\$100,000
DoD-67	Antibacterial Treatment Method Based Upon the Excretion of Dead and Decaying Spherical Bacteria	Ongoing			\$0	\$3,400,000	\$0	\$3,400,000	\$0
DoD-69	Five Year Follow-Up of Army Personnel Potentially Exposed to Chemical Warfare	Ongoing				\$946,160	\$0	\$946,160	\$0
DoD-70	War Syndromes from 1900 to the Present: Symptom Patterns and Long Term Health Outcomes	Ongoing				\$734,687	\$0	\$734,687	\$115,000
DoD-71	A Comparison of Post-Deployment Hospitalization Incidence between Vietnam and Gulf War Veterans	Ongoing	\$0	\$0	\$0	\$566,000	\$0	\$566,000	\$0
DoD-72	Long-Term Effects of Subchronic Exposure to Sarin, Alone and with Stress and Other Chemicals	Ongoing				\$996,000	\$0	\$996,000	
DoD-73	Post-Deployment Morbid Stress, Behavior and Health: Developing a Model for Predicting Morbidity, Mortality, and other Adverse Outcomes	Ongoing				\$500,000	\$0	\$500,000	\$0

DoD-74	Relationship of Stress Exposures to Health in GWVs	Ongoing				\$161,489	\$1,991,330	\$2,152,819	\$0
DoD-75	Toxic Interactions of Prophylactic Drugs and Pesticides	Ongoing					\$1,380,157	\$1,380,157	\$0
DoD-76	Evaluation of Immunotoxicity Due to Concurrent Exposure to DEET, Pyridostigmine, and JP-8 Jet Fuel	Ongoing					\$448,369	\$448,369	\$0
DoD-77	Percutaneous Absorption of Chemical Mixtures Relevant to the Gulf War	Ongoing					\$760,031	\$760,031	\$0
DoD-78	Experimental Models of Gulf War Syndrome	Ongoing					\$2,179,097	\$2,179,097	\$0
DoD-79	Military Operational Stress-Related Illnesses: Time-course of Stress-Induced Impairment of the	Ongoing				\$100,200	\$0	\$100,200	\$0
DoD-80	Molecular Regulation of Corticosteroid Receptor Expression in Stress-Responsive Cells	Ongoing				\$297,400	\$0	\$297,400	\$0
DoD-81	Immunotoxicity Due to Coexposure of DEET, PB, and Stress	Ongoing				\$300,000	\$0	\$300,000	\$0
DoD-82	Feasibility of Developing a Registry of PTSD-Affected Sibling	Ongoing				\$172,000	\$0	\$172,000	
DoD-83	Risk for Stress-Related Substance Abuse: Effects of Family History of Alcoholism	Ongoing				\$299,700	\$0	\$299,700	\$0
DoD-84	Psychobiological Alterations Of Persian Gulf War Veterans with and without PTSD	Ongoing				\$300,000	\$0	\$300,000	\$0
DoD-85	Central Nervous System Cytokines and CRH in Gulf War Veterans with Multiple Unexplained Symptoms	Ongoing				\$149,900	\$149,200	\$299,100	\$0
DoD-86	Effects of Combat Stress on the Structure and Function of the Hippocampus	Ongoing				\$300,000	\$297,800	\$597,800	\$0
DoD-87	Measurement and Validation of Psychosocial Risk and Resilience Factors	Ongoing				\$289,100	\$0	\$289,100	\$0

DoD-88	Clinical Relevance of Novel Immunological Markers in PTSD	Ongoing				\$242,300	\$0	\$242,300	\$0
DoD-89	Limbic Blood Flow and Opiate Receptor and MR Imaging in PTSD	Ongoing				\$288,500	\$0	\$288,500	\$0
DoD-90	SPECT Benzodiazepine Receptor and MR Imaging in PTSD	Ongoing				\$200,000	\$100,000	\$300,000	\$0
DoD-91	Neurological and Circadian Substrates of PTSD-Like Behaviors	Ongoing				\$300,000	\$299,000	\$599,000	\$0
DoD-92	Traumatic Experiences Enhance Cue-Dependent Learning	Ongoing				\$249,700	\$0	\$249,700	\$0
DoD-93	Follow-Up Investigation of Troops Exposed to Nerve Agents at Aberdeen Proving	Pending						\$0	\$933,000
	<i>DoD Total Funds</i>		\$6,565,814	\$11,885,000	\$11,894,940	\$29,232,536	\$12,853,325	\$72,853,325	\$22,593,000

Department of Health and Human Services Funding

<i>Project #</i>	<i>Title</i>	<i>Status</i>	<i>FY'94</i>	<i>FY'95</i>	<i>FY'96</i>	<i>FY'97</i>	<i>FY'98</i>	<i>94-98 Total</i>	<i>Projected FY'99</i>
HHS-1	Health Assessment of Persian Gulf War Veterans from Iowa	Ongoing		\$1,764,782	\$1,616,755	\$0	\$0	\$3,381,537	\$162,000
HHS-2	Disease Cluster in a Pennsylvania Air National Guard Unit, EPI-AID 95-18	Ongoing		\$750,000	\$0	\$0	\$16,055	\$766,055	\$0
HHS-3	Biomarkers of Susceptibility and Polycyclic Aromatic Hydrocarbon (PAH) Exposure in Urine and blood Cell DNA from U.S. Army Soldiers Exposed to Kuwaiti Oil	Complete	\$0	\$0	\$0	\$0		\$0	
HHS-4	Suspected Increase of Birth Defects and health Problems Among Children Born to Persian Gulf War	Complete	\$0	\$0				\$0	

	Mississippi								
HHS-5	Cognitive Function and Symptom Patterns in Gulf War Veterans	Ongoing					\$600,000	\$600,000	\$558,000
HHS-6	Defining Gulf War Illness	Ongoing					\$600,000	\$600,000	\$480,000
HHS-7	Immunotoxicity of Dermal Permethrin and Cis-Urcanic Acid	Ongoing					\$175,706	\$175,706	\$192,445
HHS-8	Strategy to Identify Non-Additive Response to Chemical Mixtures	Ongoing					\$242,586	\$242,586	\$247,933
	HHS Total Funds		\$0	\$2,514,782	\$1,616,755	\$0	\$1,634,347	\$5,765,884	\$1,640,378

Department of Veterans Affairs Funding

<i>Project #</i>	<i>Title</i>	<i>Status</i>	<i>FY'94</i>	<i>FY'95</i>	<i>FY'96</i>	<i>FY'97</i>	<i>FY'98</i>	<i>94-98 Total</i>	<i>Projected FY'99</i>
VA-1	VA Mortality Study and VA National Health Survey of Persian Gulf Veterans (combined for FY'94-97); VA Mortality Study (FY' 98 - present)	Ongoing	\$546,000	\$340,000	\$1,980,000	\$440,032	\$178,197	\$3,484,229	\$166,848
VA-2	VA National Survey of Persian Gulf Veterans	Ongoing						\$0	
VA-2A	VA National Survey of Persian Gulf Veterans - Phase I	Complete					\$18,111	\$18,111	
VA-2B	VA National Survey of Persian Gulf Veterans - Phase II	Ongoing					\$271,551	\$271,551	\$0
VA-2C	VA National Survey of Persian Gulf Veterans - Phase III	Ongoing					\$1,601,280	\$1,601,280	\$2,340,774
VA-3	Use of Roster of Veterans Who Served in Persian Gulf Area	Complete					\$0		
VA-4	VA National Survey of Persian Gulf Veterans - Phase IV	Ongoing	\$98,300	\$500,000	\$500,000	\$500,000	\$500,000	\$2,098,300	\$500,000

	Hazards Research								
VA-4A	Evaluation of Cognitive Functioning of PGWVs	Ongoing						\$0	
VA-4B	Evaluation of Neurological Functioning in Persian Gulf Veterans (see new HHS-5)	Complete						\$0	
VA-4C	Gulf War And Vietnam Veterans Cancer Incidence Surveillance	Ongoing						\$0	
VA-4COR	Boston Environmental Hazards Research Center - Core	Ongoing						\$0	
VA-4D	Evaluation of Respiratory Dysfunction Among Gulf War	Ongoing						\$0	
VA-4E	The Aromatic Hydrocarbon Receptor (AhR) as a Biomarker of Susceptibility	Ongoing						\$0	
VA-4F	Validity of Computerized Tests	Ongoing						\$0	
VA-5	East Orange Environmental Hazards Research Center	Ongoing	\$100,000	\$500,000	\$500,000	\$500,000	\$500,000	\$2,100,000	\$500,000
VA-5A	Health and Exposure Survey of Persian Gulf Veterans	Ongoing						\$0	
VA-5B	Physiological and Psychological Assessments of Persian Gulf Veterans	Complete						\$0	
VA-5C	Effects of Exertion and Chemical Stress on Persian Gulf Veterans	Ongoing						\$0	
VA-5COR	East Orange Environmental Hazards Research Center - Core	Ongoing						\$0	
VA-5D	Effects of Genetics and Stress on Responses to Environmental Toxins	Complete						\$0	
VA-6	Portland Environmental Hazards Research	Ongoing	\$99,279	\$499,583	\$498,695	\$499,198	\$499,926	\$2,096,681	\$500,000

	Veterans Health and the Gulf War Syndrome								
VA-6A	Psychosocial, Neuropsychological and Neurobehavioral Assessment (Project I)	Ongoing						\$0	
VA-6B	Clinical and Neuroendocrine Aspects of Fibromyalgia (Project II)	Ongoing						\$0	
VA-6C	Neurotoxicity of Environmental Pollutants and Warfare Agents (Project III)	Ongoing						\$0	
VA-6COR	Core: Portland Environmental Hazards Research Center: Environment, Veterans Health and the Gulf War Syndrome. Core Project for Clinical and Epidemiology Research	Ongoing						\$0	
VA-6D	DNA Damage from Chemical Agents and Its Repair (Project IV)	Ongoing						\$0	
VA-6E	Clinical and Epidemiology Leishmania Research	Ongoing						\$0	
VA-7	Desert Storm Reunion Survey	Complete	\$0	\$50,000				\$50,000	
VA-8	Psychological Test Data of Gulf War Veterans Over Time	Ongoing	\$0	\$0	\$0	\$0	\$0	\$0	\$0
VA-9	Evaluation of Cognitive Functioning in Persian Gulf War Veterans Reporting War-related Health Problems	Complete	\$0	\$0	\$20,000			\$20,000	
VA-10	Memory and Attention in PTSD	Ongoing	\$0	\$0	\$63,700	\$57,000	\$57,600	\$178,300	\$0
VA-11	Neuropsychological Functioning in Veterans	Complete	\$0	\$0				\$0	
VA-12	Psychological Assessment of Operation Desert Storm	Complete	\$0	\$0	\$0	\$0		\$0	

VA-13	Neurobehavioral Aspects of Persian Gulf Experiences: A Pilot Study	Complete	\$0	\$50,000				\$50,000	
VA-15	Vaccine-Mediated Immunity Against Leishmaniasis	Ongoing	\$64,300	\$64,300	\$0	\$82,600	\$80,000	\$291,200	\$79,400
VA-16	Protective Immunity in Experimental Visceral Leishmaniasis	Complete		\$60,200	\$60,700	\$54,900		\$175,800	
VA-17	Immunological Evaluation of Persian	Complete	\$0	\$0				\$0	
VA-18	Chronic Gastrointestinal Illness in Persian Gulf Veterans	Complete		\$0	\$0			\$0	
VA-20	Psychological Adjustment in Operation Desert Shield/Storm	Complete						\$0	
VA-21	A Comparison of PTSD Symptomatology among Three Army Medical Units Involved in ODS	Complete	\$0	\$0				\$0	
VA-36	Stress Symptoms and Their Causal Attribution in Desert Storm Veterans	Complete		\$0	\$0	\$0		\$0	
VA-40	Musculoskeletal Symptoms in Gulf War Syndrome	Ongoing	\$0	\$0	\$0	\$0	\$0	\$0	\$0
VA-46	Diarrhea in Persian Gulf Veterans: An Irritable Bowel-Like Disorder	Ongoing		\$0	\$0	\$0	\$0	\$0	\$0
VA-47	Retrospective Verification of Mustard	Ongoing				\$37,300	\$37,300	\$74,600	\$37,300
VA-48	Cross-Sensitization as a CNS Model for Gulf War Chemical Intolerance	Ongoing					\$99,900	\$99,900	\$98,800
VA-49	Sensitivity to Pyridostigmine Bromide: Persistent Neural Dysfunction	Ongoing					\$184,400	\$184,400	\$13,300
VA-50	Neuropsychological Function in Gulf War Veterans	Complete		\$0	\$0	\$0		\$0	
VA-51	Psychobiological	Ongoing	\$0	\$0	\$0	\$0	\$0	\$0	\$0

	Storm Veterans								
VA-53	Spouses and Children Program	Ongoing				\$101,360	\$98,651	\$200,011	\$1,799,989
VA-54	Follow-up of Psychological and Neurocognitive Gulf War Outcomes: Relation to Stress	Ongoing						\$0	\$102,000
VA-55	Antibiotic Treatment of Gulf War Veterans' Illnesses	Ongoing					\$50,000	\$50,000	\$2,000,000
	VA Total Funds		\$907,879	\$2,064,083	\$3,623,095	\$2,272,390	\$4,176,916	\$13,044,363	\$8,138,411

VA/DoD Programs Funding

<i>Project #</i>	<i>Title</i>	<i>Status</i>	<i>FY'94</i>	<i>FY'95</i>	<i>FY'96</i>	<i>FY'97</i>	<i>FY'98</i>	<i>94-98 Total</i>	<i>Projects FY'99</i>
VA/DoD-1D	VA Cooperative Study 470: A Randomized, Multi-Center, Controlled Trial of Multi-Modal Therapy in Veterans with Gulf War Illnesses (GWIs)						\$4,500,000	\$4,500,000	
VA/DoD-1V	VA Cooperative Study 470: A Randomized, Multi-Center, Controlled Trial of Multi-Modal Therapy in Veterans with GWIs	Ongoing					\$50,000	\$50,000	\$2,500,000
VA/DoD-2D	VA/DoD Core Funding of the Medical Follow-Up Agency	Ongoing	\$250,000	\$250,000	\$250,000	\$250,000	\$250,000	\$1,250,000	\$250,000
VA/DoD-2DA	Follow-Up Investigation of Troops Exposed to Nerve Agents at Aberdeen Proving Ground (Pilot Study)							\$0	
VA/DoD-2DB	Patterns of Pre-Persian Gulf War Illness and Health Care Seeking - Pilot Study							\$0	
VA/DoD-2V	VA/DoD Core Funding of the Medical Follow-Up Agency	Ongoing	\$250,000	\$250,000	\$250,000	\$250,000	\$250,000	\$1,250,000	\$250,000

VA/DoD-2VA	Follow-Up Investigation of Troops Exposed to Nerve Agents at Aberdeen Proving Ground (Pilot Study)	Complete						\$0	
VA/DoD-2VB	Patterns of Pre-Persian Gulf War Illness and Health Care Seeking-Pilot Study	Complete						\$0	
	<i>VA/DoD Total Funds</i>	\$500,000	\$500,000	\$500,000	\$500,000	\$5,050,000	\$7,050,000	\$3,000,000	